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The reintroduced Dinaric lynx population dynamics in PVA simulation,

The 30 years retrospection and the future viability

Modeli razvoja in analiza viabilnosti re-introducirane dinarske populacije risa,

30-letni pogled nazaj ter možnosti preživetja v prihodnosti

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Abstract. In the study, we modelled population dynamics of the reintroduced Dinaric lynx population. We used data obtained by monitoring to estimate population dynamics – spatial expansion, abundance estimates, and mortalities – since the reintroduction in 1973 and up to the present day, and then looked for demographic and habitat parameters that would provide the best fit of a lynx population model to this data. We tried to evaluate the importance of these parameters for future population dynamics and viability (PVA) of this lynx population. We constructed a number of 100-year simulations using a range of demographic parameters, different prey availabilities and simulating other potential human related factors that might affect the lynx population. We found that the reintroduced lynx population must have had high fecundity rates with more than 1.6 kittens survived per female and per litter to reach abundances over 100 individuals despite the high human related mortality. The elasticity analysis revealed that adult survival is by far the most important demographic parameter for the lynx population dynamics. PVA highlighted two important factors that had a major impact on population growth dynamics and related risk of population extinction: changes in the survival rates of subadult and adult individuals and, especially, the quality of habitat with regard to prey availability. Survival rates of subadult and adult lynx are directly influenced by human activities, mainly manifested through illegal shooting, and are difficult to control. Quite opposite to that, the quality of habitat with regard to prey availability can be directly influenced through management. Since habitat quality can have a significant role for the lynx population dynamic and viability, even in presence of minor, difficult to control changes in survival rates of subadults and adults, adequate prey species management might be one of the most important short-term conservation priorities.

Keywords. Eurasian lynx, *Lynx lynx*, population dynamics, PVA simulation, reintroduction, Dinaric region

Izvleček. V raziskavi smo zgradili modele populacijske dinamike ponovno naseljene populacije risa v Dinaridih. Pri oblikovanju modelov smo uporabili podatke, ki so bili pridobljeni pri spremljanju razvoja populacije od leta 1973 kot so: prostorsko širjenje, ocena številčnosti in smrtnost ter nato poiskali ustrezne demografske in prostorske parametre s katerimi smo dobili populacijski model, ki v največji meri ustreza zbranim podatkom. Poskušali smo oceniti pomen teh parametrov za razvoj in viabilnost populacije v prihodnje. Pripravili smo serijo simulacij 100 letne populacijske dinamike risa pri čemer smo variirali demografske parametre, razpoložljivost plena ter odsimulirali druge antropogene dejavnike, ki bi lahko vplivali na populacijo risa. Ugotovili smo, da je morala biti stopnja rodnosti ponovno naseljene populacije visoka z več kot 1.6

preživelih mladičev na samico. Le tako bi lahko populacija dosegla številčnost preko 100 osebkov ob hkratni visoki antropogeni smrtnosti risov. Analiza elastičnosti populacije je pokazala, da je preživetje odraslih osebkov daleč najpomembnejši demografski parameter, ki v največji meri vpliva na populacijsko dinamiko. Z analizo populacijske viabilnosti (PVA) smo ugotovili dva pomembna dejavnika, ki imata največji vpliv na dinamiko populacijske rasti in z njo povezana tveganja za izumrtje populacije. To sta preživetje doraščajočih in odraslih osebkov ter kvaliteta risovega habitata z vidika razpoložljivosti (gostote) plena. Na spreminjanje stopnje preživetja ima največji vpliv človek s svojim neposrednim vplivom (zakoniti in nezakoniti lov, promet), ki ga je težko regulirati. Ravno nasprotno pa lahko upravljalci z ustreznim upravljanjem s plenskimi vrstami v veliki meri vplivajo na kvaliteto risovega prostora z vidika dostopnosti hrane. Zato je lahko zadnje eden izmed najpomembnejših kratkoročnih varstvenih ukrepov pri upravljanju s populacijo risa v Sloveniji v prihodnje.

Ključne besede. Evrazijski ris, *Lynx lynx*, populacijska dinamika, PVA simulacija, reintrodukcija, dinarska regija

Introduction

The reintroduction of individual animals of a certain species to an area where the species has become extinct in the recent past has become one of the most important, albeit controversial, approaches to conservation of threatened species over the last few decades (Breitenmoser & al. 2001, Bekoff 2001). The current presence of the Eurasian lynx (*Lynx lynx*) in Slovenia is a direct result of a successful reintroduction. The reintroduction project was started in 1972, when experiences with carnivore recovery programs were still very limited. Considering the contemporary requirements that such reintroduction projects should meet (IUCN/SSC, 1998), the reintroduction program had many deficiencies, especially from valuational and organisational point of view. However, the reintroduction was performed with consent and cooperation of hunters, the most important interest group that had the power to directly affect the success of the reintroduction. This was of great importance, since review analyses show that in successful reintroductions a human – carnivore conflict either did not exist, or had been reasonably solved (Reading & Clark 1996, Wolf & al. 1996, Breitenmoser & al. 2001). Besides, the newly established population had favourable habitat and prey conditions (Čop 1994), which can be considered of key importance for the reintroduction's success. Since only three pairs of lynx were introduced, there can be no doubt that the population experienced the founder effect through the genetic drift (Breitenmoser-Würsten & Obexer-Ruff 2003), a reduction of heterozygosity, a loss of alleles and a further depletion of its gene pool through inbreeding. These negative predispositions aside, the monitoring of the population shows that the population has undergone a rapid expansion into Croatia and Bosnia and Herzegovina, and also expansion towards the north into Austria and towards the northwest into Italy. The long term monitoring effort has been initiated soon after the reintroduction, and several comprehensive papers and internal reports have been published (eg. Čop 1994, 1997, Čop & Frković 1998, Frković 2003, Staniša & al. 2001, Jonozovič 2004). However, the monitoring has been mainly oriented in collection of spatial distribution of lynx signs of presence and on recording of killed (culled) lynxes. According to these, the expansion into the unoccupied areas came to a halt in the early nineties.

Although the monitoring effort is very important, other population studies should also be considered. Telemetry (Huber & al. 1995), population modelling and population viability analysis (PVA) may prove to be of great value for management of the Dinaric lynx population, since we still cannot be confident in its long-term survival.

In general, population viability analysis is a useful tool for identification of variables important for population growth and the resulting viability of reintroduced or other small populations. We performed the analysis in two parts. In the first part, we used the monitoring data to estimate population dynamics of the Dinaric lynx population (spatial expansion, abundance estimates, and mortalities) since the reintroduction up to the present day, and then looked for demographic and habitat parameters that would provide the best

fit of a lynx population model to this data. We applied different scenarios in modelling of population dynamics of the re-introduced lynx from 1973 to 2003, using different demographic and/or environmental parameters. In the second part of the analysis we tried to evaluate the importance of the same parameters for future population dynamics and viability (PVA) of such a lynx population. We constructed a number of 100-year simulations using a range of demographic parameters, different prey availabilities and simulating other potential human related factors that might affect the lynx population.

Methods

PVA software and model parameters

The data on demographic attributes for the Dinaric lynx population are scarce, if not non-existent. To be able to model the development of the Dinaric lynx population, we estimated its population parameters from knowledge of lynx biology from other parts of the species' range.The simulations were performed using the RAMAS Metapop® software package (Applied Biomathematics, USA). All analyses and models were produced for the Dinaric lynx population as a whole and in its entire geographic range (Slovenia, Croatia and Bosnia and Herzegovina), since doing population dynamics and viability for individual parts of the population doesn't make much sense. Each scenario used for modelling of the population dynamics was run with 1000 iterations. The birth sex ratio used for the modelling was 1:1, since the sex structure in stable lynx populations shows a balanced ratio between males and females (Kvam 1991). We designed a post-reproductive Leslie matrix for both, males and females in seven separate stages, three for females and four for males. For both, we applied three age categories: yearlings, subadults and adults. Male subadults were placed in two stages. Although they can become sexually mature at the end of their second year of life, it is unlikely for them to breed until the end of their third year. The age at first reproduction is the demographic parameter that significantly affects the population dynamics (Akcakaya 2002). Histological analysis of testicles in the Norwegian lynx population has shown that 50% of males reach sexual maturity at the age of 21 months (Kvam 1990). However it is unlikely that young males would participate in mating, since they are being chased away from females by older males. This probably means that they can mate only a year later, when they are almost 3 years old (Kvam 1991). The females in captivity reach sexual maturity at 21 months (Lindeman 1955, Henriksen & al. 2005). A similar result was obtained in Norway through analysis of ovulation or presence of corpora lutea, where most females also reached sexual maturity by the age of approximately 21 months. However, roughly half of the females were sexually mature already at the age of 7.5–11.5 months (Kvam 1990).

Reproductive success of a lynx population depends primarily on the feeding conditions in the environment, usually the density of roe deer, *Capreolus capreolus,* (Jedrzejewski & al. 1996, Okarma & al. 1997). When they become sexually mature, Eurasian lynx females usually mate until the high age of 12–13 years (Stehlik 1984, Stahl & Vandel 1998, Henriksen & al. 2005). In a radio-telemetric study in Scandinavia they found that 55% of two-year old females and 72% of older females were with kittens (Andren & al. 1998, 2002). In the model, we defined this proportion of females as reproductively active. Subadult (1 year old) females were defined as non-reproductive.

Many European studies of lynx reproduction found that the number of kittens per female at the end of their first year of life was between 1.6 and 1.2 (Kvam 1991, Pulliainen 1995, Jedrzejewski & al. 1996, Andren & al. 1998, 2002, Okarma & al. 1997, Jedrzejewska & Jedrzejewski 1998, Andersen & al. 2003, Henriksen & al. 2005). Deviating from this are data from Switzerland, where they found only 0.69 kittens per female in the studied animals (Breitenmoser & al. 1993). Reasons for this may be in different methodologies used to assess recruitment, or due to lower survival of kittens. We chose to use fecundities $F = 1.2$ and $F = 1.6$ for the model, referred to as the high and the low reproduction. In RAMAS Metapop®, fecundity is defined as the number of offspring that survive to the end of their first year of life per female.

Pre-dispersal and dispersal mortalities have been researched for many felid species, and they seem to vary between species (Breitenmoser & al. 1993). Survival rates of dispersing or subadult lynx in Europe range from 36 % to 62 % (Stehlik 1984, Breitenmoser & al. 1993, Andren & al. 1998, Henriksen & al. 2005). Although there are some data on survival rates of resident lynx in Europe (Jedrzejewski & al. 1996, Kramer-Schadt & al. 2005, Andren & al. 1998, 2006), the rather low statistical power of the data is common for all the studies. For the model, we used survival rates of subadult and adult lynx obtained from the survival data of 245 animals radio-tracked in the Scandinavian Lynx Project (Andren & al. 1998, 2006). The survival rates used were 62% for subadult males and females (one to two years old), 81% for two to three years old females and 83% for the males of the same age.

We are fully aware that these values might be biased due to small sample size and the limited time of the study. Additionally, these data are from the boreal region found in the northern part of the lynx areal in Europe, and we are using them to model population dynamics in a mountainous region of mixed forests in the southern part of the species' areal. However, all the values used in the model are rather high, since they should represent only background survival rates without the influence of human-related mortality which was modelled separately. We must consider that population models and PVA require information on many variables that are difficult to estimate for low-density populations (Beissinger & Westphal 1998).

Even the simplest deterministic models need information regarding age structure, breeding schedule, and vital rates (McKelvey & al. 2000). These models assume that demographic rates are constant, which is certainly not true for lynx. Small populations are more sensitive to stochastic events, which usually make them more vulnerable to various detrimental factors and can significantly decrease their viability. Demographic and environmental stochasticity were included in our model to simulate variation in population size under natural conditions. Accordingly, we modelled lynx population dynamics stochastically by generating random annual lynx vital rates from the normal distribution curve according to the values defined above, using the coefficient of variability (*CV*) of 0.2 for fecundities and 0.1 for survival rates, which covers a substantial part of the variation in lynx vital rates known from the literature considered above.

Modelling historical 30-year population dynamics

In January 1973, three adult male and three adult female lynx were transferred to a quarantine enclosure in Slovenia from a quarantine enclosure in the Stromovka Zoo near Ostrava (in today's Slovak Republic). After 46 days they spent there separated in pairs, the lynx were released into the wild. According to direct observations and observations of signs of presence (Čop 1994), none of the animals exhibited homing behaviour. Snow tracking of presumably all six animals was reported in November 1973. Thus, as the initial population size, three adult females and three adult males were modelled in our model.

In the years that followed the population grew and expanded. The initial development of the population showed a low mortality of individuals (Kos & Krofel 2004). We can assume this to be of key importance for survival of the newly established population through the initial phase (Kos & Krofel 2004). The population continued to grow despite legal culling of lynx being introduced in 1978, and also expanded in the northwestern direction towards Italy and the Alps (Čop 1994). It seems that the rapid spatial expansion of the population that took place until the middle of the eighties of the previous century slowed down by the end of the decade, and came to a halt in the beginning of the 90s (Čop 1997, Čop & Frković 1998, Frković 2003). To be able to model the development of the population, we had to determine the population ceiling (*K*). The ceiling in RAMAS Metapop® is the population size above which all individuals are killed or have died, and it does not act like usually an environmental carrying capacity would act. We defined the ceiling as the population size at its highest point in the last 30 years, which was most likely reached at the end of the spatial expansion (17 to 18 years after reintroduction).

Effects of recorded human-related mortality

Two scenarios were applied for simulation of the thirty year population growth of the Dinaric lynx population using the ceiling growth model: (1) a population with high reproduction ($F = 1.6$) and (2) a population with low reproduction ($F = 1.2$). Deterministic growth rate (lambda) and elasticity analysis were performed for each of the fecundity values. Additionally, human-related mortality was included as a relative decrease of annual vital rates, determined as a percentage of killed lynx with regard to the mean population sizes in particular years with no additional mortality modelled. Thirty percent of the decreases were modelled as decreases of fecundities, and 70% as decreases of survival rates. This corresponds to the ratio between kittens and older animals killed in this population between 1973 and 2003 (Frković 2003). During this period, there are 375 lynx recorded as harvested, poached, and killed in traffic accidents or found dead in Slovenia, Croatia, and Bosnia and Herzegovina (Potočnik 2004).

Effects of prey density

Following conclusion of the spatial expansion, and re-adaptation of the prey to the new predator, is the second phase of population dynamics, with a decrease in population density and the end of population growth (Breitenmoser & Haller 1987, 1993). In this phase, if there are no other direct factors like hunting and poaching, the density of prey becomes the most important factor, affecting mainly the survival rate of the young, and trough it directly the reproductive success of the lynx population (Brand & Keith 1979, Kvam 1991, Jedrzejewski *&* al. 1996, Okarma *&* al. 1997, Fuller & Sievert 2001, Steury & Murray 2004). Although there are a number of carnivore species, especially those with cyclical populations, like for example the Canadian lynx (*Lynx canadensis*) for which we have a good understanding of the effects the food/prey has on demography (e.g. McCord & Cardoza 1982, Breitenmoser *&* al. 1993, Slough & Mowat 1996, O'Donoghue *&* al. 1997, Mowat *&* al. 2000), such knowledge is surprisingly poor for the Eurasian lynx. Data exist from Poland, where a deliberate reduction of roe deer (35 – 40%) and red deer, *Cervus elaphus,* (30%) densities in Bialowieza forest by game managers resulted in a 50% reduction of the reproductive success of the lynx (Okarma *&* al. 1997, Jedrzejewska & Jedrzejewski 1998).

Effects of illegal hunting

The lynx has never been protected in Bosnia and Herzegovina. In the Serbian Republic of Bosnia and Herzegovina it used to be listed as a protected species between 1994 and 2001, but the effectiveness of the legal protection in the post-war political situation is questionable. According to reports, lynx are hunted whenever seen (Soldo 2001). Until 2001, 25 lynx have been killed, with the first lynx killed in 1983 (Soldo 2001). This represents 7% of all killed lynx reported for the entire Dinaric population. We can assume that Bosnia and Herzegovina represents a sink area with a low population density. In 1993, Slovenia passed a Decree on the Protection of Endangered Animal and Plant Species, and the lynx received the status of a species under strict protection. In 1998 it received a complete legal protection in Croatia as well. A year before that a new Hunting Act was passed in Croatia, introducing a leasing system for hunting grounds and effectively making hunting primarily an economic activity. Because of the complete legal protection the lynx lost its trophy value for hunters, which was possibly an important mechanism of the so-called active protection. All this made predators, especially the large carnivores, indirectly characterized as economic pests. A sentiment that there is no influence or control over the population size of a predator, and consequently no control over the effects it has on the prey species, certainly bears negative consequences for conservation of the predator. This is most often manifested through an increase of illegal hunting of the predator (Breitenmoser *&* al. 2001, Červeny 2002). Illegal shooting of lynx is a considerable mortality factor and a threat to a small population (Breitenmoser *&* al., 1998). The data on illegal hunting or poaching of lynx in Switzerland and Poland confirm this, as poaching in Poland contributed to 75% of all known lynx mortality and posed the most important mortality

factor in Switzerland (Breitenmoser & al. 1993, Schmidt-Posthaus & al. 2002, Jedrzejewski & al. 1996, Okarma & al. 1997).

Prey density and additional mortality as important factors for future viability

As a theoretical starting point for the Dinaric lynx PVA, we can use the estimate that the current lynx population numbers 100 individuals that live in an area of 20,000 km^2 (Frković 2003, Fišt & al., 2003, Kos & al., 2004). The initial population size used in the model was 100 individuals, with a stage structure equal to the calculated stable stage structure rounded to the closest integer. The population was limited just above the initial population size with a ceiling at $K = 110$ ($SD \pm 20$). Allee effects in RAMAS Met&apop® were determined by the function $N/(N+A)$, representing a relative decrease in fecundity with regard to the population size. A value of $A = 2$ was chosen, which decreased the vital rates for more than 5% when the population fell below the density of 1 individual per 500 km^2, which was double the size of an adult male home range radio-tracked in the region (Huber & al. 1995).

Results

Modelling historical 30-year population dynamics

We ran simulations of an exponential population growth for 17 and 18 model years. We included effects of recorded human-caused mortality (Čop 1997, Čop & Frković 1998, Frković 2003, Potočnik 2004), modelled as a removal of the specific number of individuals per each year. The simulated population reached mean sizes between 90 and 130 individuals. Therefore, we determined the population ceiling at $K = 110$ with a standard error (SE +/– 20) to simulate variation due to environmental stochasticity. These results are supported by estimations from the population monitoring reports of Slovenian Forest Service and Frković (2003). Judging by the results of simulations of potential inbreeding coefficients expected in the Dinaric lynx population 30 years after the reintroduction (Potočnik 2005), the inbreeding depression is not expected to have an important role in population dynamics during that period. However, for the simulations the founder animals were assumed unrelated and with a random choice of reproductive mates which is unlikely. So far there has been no evidence of the inbreeding depression (beside population decline), so we didn't include it in the models. However, since there is definitely inbreeding in the population, and there is recent genetic data to confirm it (Majić & al. unpubl.), this factor should be included in future modelling exercises as it can soon become of major importance for viability of the Dinaric lynx population.

Effects of recorded human-related mortality

Values obtained for lambda at high and low reproduction were 1.2 and 1.15, respectively. The elasticity analysis showed survival of adult individuals to be the most important parameter, with proportional contributions to population growth rates of 0.46 and 0.49 (Table 1).

Simulations showed that the modelled population with high reproduction would grow relatively fast until the year 1991. Afterwards, the population growth slows down, reaching

Table 1: Elasticity, the relative contribution of demographic parameters to the population growth.

Tabela 1: Elastičnost demografskih parametrov modela populacijske dinamike risa pri visokem oziroma nizkem reprodukcijskem uspehu.

Demographic parameter	Elasticity	
	high reproduction $F = 1.6$	low reproduction $F = 1.2$
fecundity (kitten recruitment)	0.19	0.18
survival of 1 - 2 year old individuals	0.17	0.16
survival of 2 - 3 year old individuals	0.18	0.17
survival of adults	0.46	0.49

the ceiling in 2003 (Figure 1). As a contrast, the population with low reproduction would grow slowly until 1991, and would not reach

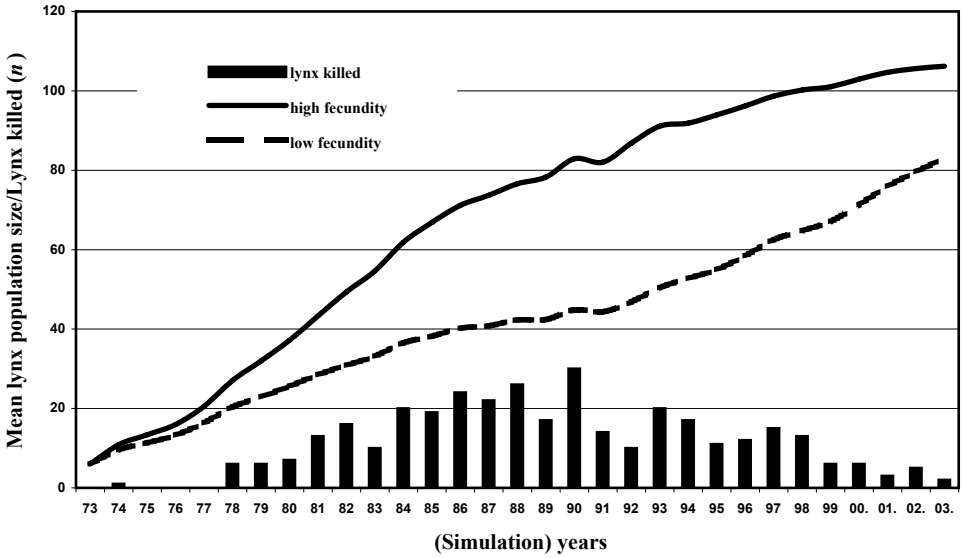

Fig. 1: Stochastic population growth models over a 30–year period with an initial population of three adult females and three adult males, taking into account the recorded lynx mortality.

Slika 1: Stohastični modeli populacijske dinamike risa v 30–letnem obdobju z začetno populacijo treh odraslih samcev in treh samic, upoštevajoč vpliv znanih izgub na celotnem območju.

the ceiling within 30 years despite faster growth in the years that follow. Thus, the population dynamics of the population with high reproduction was closer to the dynamics observed in the monitoring reports, and although the model did not include some mechanisms that are important for reintroduced populations, it was retained for further analysis.

These mechanisms were studied during expansion of a reintroduced lynx population in Switzerland (Breitenmoser & Haller 1987, Haller 1992, Breitenmoser & al. 1993). The mechanisms affecting population dynamics of a reintroduced lynx population can be divided into two phases. The dispersion of young animals is an important factor in the first phase of population growth, enabling a high survival rate and rapid spatial expansion of the population. It is a simple mathematical phenomenon, additionally amplified by naive prey that is not adapted to the new predator (Breitenmoser & Haller 1987, Haller 1992). We can assume that the first phase, the rapid expansion of the Dinaric lynx population, took place until the end of the eighties or the beginning of the nineties of the previous century, when it slowed down or stopped.

Effects of prey density

To simulate the effects of prey density and availability on reproductive success of the Dinaric lynx population, we included the data on roe deer harvest in the model as a prey density parameter for the years after 1993, when the lynx expansion presumably stopped. We can assume that the harvest of roe deer population decreased with a decrease in roe deer population. The reasons for the decrease are various, including the effects of the presumable increase of the wolf population as well as the significant change in sex structure of the harvest with a much larger proportion of females being harvested. According to the Slovenian Forest Service and the Slovenian Hunters Association's harvest data, there was 40% decrease in the roe deer population size

between 1993 and 2003. Following the results from Bialowieza, we modelled the effects of prey density as a reduction in fecundity relative to the decrease in the harvest in a particular year with regard to the harvest in 1993. The maximum reduction of fecundity was down to 60% of the initial fecundity when the reduction of roe deer population was 40%. Simulations of population 1 to 10% and from 1 to 15% (Figure 2). In both simulations the populations started declining in a year when progressive effect of illegal hunting reached a 5% decrease in survival rates of all age and sex categories (Figure 2). Obtained mean population sizes at the end of simulations were 81 and 91 lynx, respectively. On basis of the available lynx monitoring data the Slovenian Forest

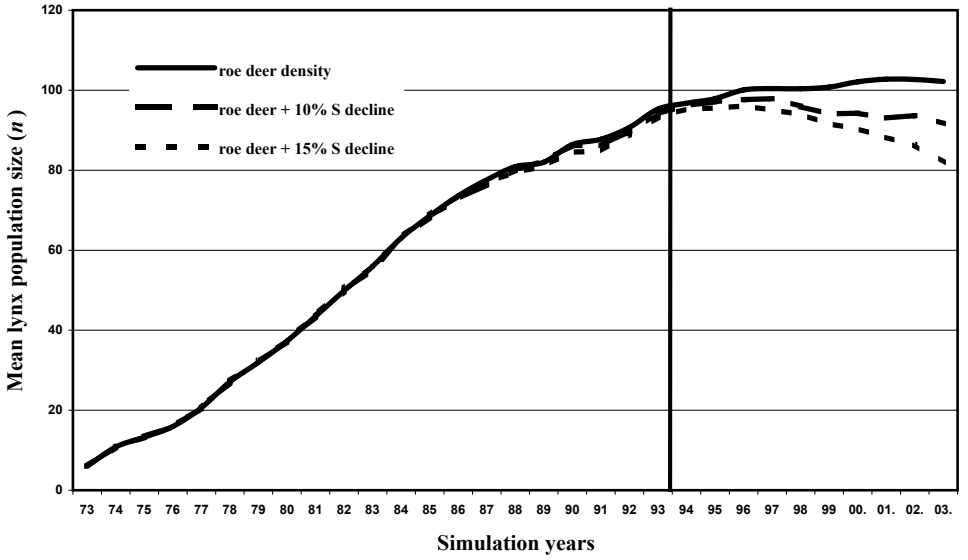

Fig. 2: Stochastic lynx population growth models over a 30–year period with high reproduction, taking into account changes in roe deer abundance (roe deer density) and a gradual decrease in survival rates (S decline) caused by increased human related mortality in the last 10 years.

Slika 2: Stohastični modeli populacijske dinamike risa v 30–letnem obdobju ob visoki reprodukciji, upoštevajoč spreminjanje številčnosti srnjadi (roe deer density) in postopno zmanjševanje preživetvene stopnje (S decline) risov zaradi povečane antropogene smrtnosti v zadnjih 10. letih.

dynamics that included the effects of prey density showed that the population would stop growing after 1993 at a mean population size of approximately 100 individuals (Figure 2).

Effects of illegal hunting

In contrast to the prey density, the illegal hunting is very difficult to measure or estimate. However, we assume that the strict protection of the lynx and the new hunting legislation in Slovenia and Croatia have caused a progressive increase in illegal hunting of this species. The effect was modelled, in addition to the prey density effect, as a 10–year linear decrease of survival rates from

Service estimated the number of lynx in Slovenia in 2003 to be approximately 45 individuals. The estimate for Croatia is 50 individuals (Frković 2003, Firšt & al. 2003). The data available on the recent population status of lynx in Bosnia and Herzegovina are scarce. Since the number of recorded dead lynx is low (% of all recorded losses in the Dinaric population) despite extremely relaxed hunting regulations, we believe the estimate of 40 individuals, from the country report (cf. von Arx & al. 2004) to be quite exaggerated. With all that in mind and, despite the constructed model is being a simplified description of relations in the real population, we assume that it can be used as

a helpful basis for population viability analysis for the Dinaric lynx population.

Prey density and additional mortality as important factors for future viability

In simulations of population dynamics and viability analysis for the next 100 years, we exposed the population to different situations in the environment: (1) an environment with high prey density, simulated as high fecundity (F = 1.6), (2) an environment with medium prey density, simulated as medium fecundity (F = 1.2, –25%), and (3) an environment where prey density is low, simulated as low fecundity (F = 1.0, –40%). In all three cases we studied the effects of the decrease in lynx survival rates resulting from additional mortality (Figure 3) on the associated risks of population extinction over a 100-year period (Figure 3). To determine the acceptable, non-detrimental level of additional mortality, in the model considered as the effect of culling (illegal shooting), the population was considered viable if the risk of its extinction over a 100 years period was below 5% (Shaffer 1981).

In different prey density scenarios, the simulations showed substantial differences in the decline of survival rates. There was no extinction risk in any of the scenarios when no additional mortality was included in the model. That means that in the absence of additional mortality a decline in fecundities as severe as 40% would not pose a threat to the Dinaric lynx population. On the other hand, the prey density had a drastic influence on the extinction risk dynamics in presence of progressive decreases of survival rates. In the high prey density scenario, the limit of the population viability was reached at a 10.5% decrease in survival rates. On the contrary in the medium and the low prey density conditions the risk of extinction reached the threshold of viability at 6% and 3%, respectively (Figure 3). Results of the model indicate that the population is much more sensitive to even minimal changes in survival rates of adults and subadults (as a result of additional human related mortality) under the low prey density conditions than when the prey density is high.

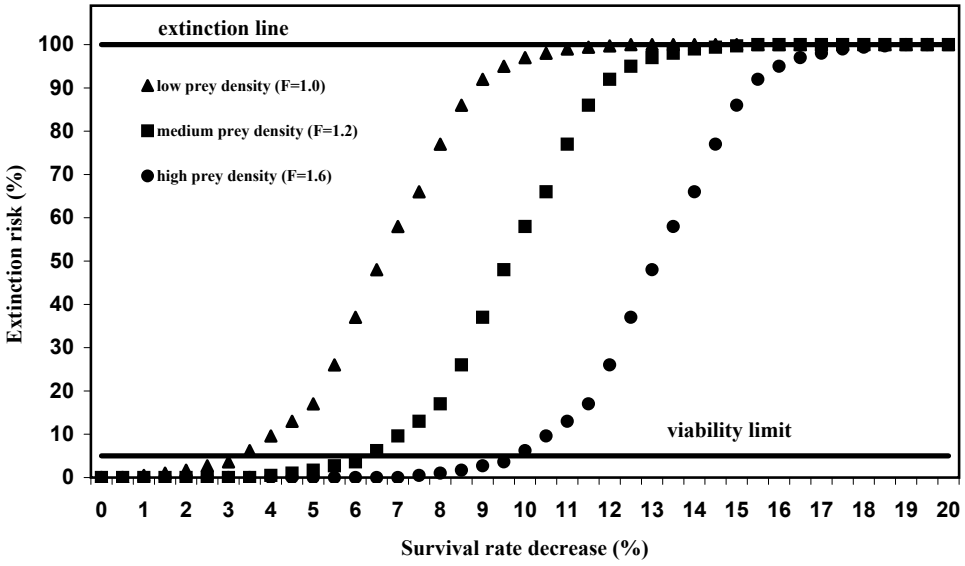

Fig. 3: Extinction risk dynamics influenced by decreasing survival rates caused by human related mortality in subadult and adult lynx at different fecundities, simulating different prey densities.

Slika 3: Dinamika tveganja za izumrtje populacije ob zmanjševanju stopnje preživetja doraščajočih in odraslih risov, kot posledice povečane antropogene smrtnosti ob različni reproduktivni uspešnosti s katero smo simulirali različno gostoto plena v okolju.

Discussion

Carnivores, especially lynx, are elusive animals, and to monitor the progress and the success of a reintroduction programme is a difficult, expensive and long lasting task (Breitenmoser & al. 2001). There is a clear need to improve the efficiency of reintroductions. By simulating population dynamics and viability of the Dinaric lynx population we tried to contribute to understanding of the reintroduction mechanisms. The thirty year retrospective models simulating the Dinaric lynx population growth indicate that the reintroduced population must have had high fecundity rates (F > 1.6) to reach abundances over 100 individuals despite the high human related mortality. The high human related mortality, especially in Bosnia and Herzegovina, might be one of the reasons for the slow down of lynx expansion (von Arx & al. 2004) or probably low dispersion to the southern Dinaric Mountains. An expansion toward north and northwest into the Alps in Slovenia has been probably slowed down due to significant spatial obstacles (traffic infrastructure, urban areas, open habitat) separating the Alps from the Dinaric region (Skrbinšek 2004). In Slovenia, no reproduction has been recorded in the Alps (Staniša &al. 2001). We can assume these obstacles to have an important effect on local oscillations in population size and may obstruct the population flow. This would have a d&rimental effect on viability of the entire population and increase the risk of local extinctions. The effect of the matrix obstacle increases when a dispersion pressure from the core area of the population is decreased.

Approximately 20 years after the reintroduction, when the intensive expansion of the population into new, unoccupied habitats came to an end, the population probably culminated and habitat quality parameters, like the prey density, became important density dependant factors. Changes in reproductive output are certainly a major potential response, and the relationship of nutrition and both reproduction and recruitment is well documented in mammals (Sadleir 1969). Since recruitment to a population is a function of the proportion of productive females, litter size, and offspring survival, these demographic parameters should be higher when prey is relatively abundant (Fuller & Sievert 2001). Changes in food abundance can drastically affect the survival of new-borns. Post-partum mortality of Canadian lynx kittens during a snowshoe hare, *Lepus americanus,* population decline was the main factor responsible for a lack of recruitment to the lynx population in Alberta (Brand & Keith 1979). A slower increase of home-range sizes with a decrease in prey density in female than in male lynx (Herfindal & al. 2005) might be, as an alternative to the Sandell's (1989) prediction of a change in the mating tactics of males, explained by a decreased reproductive output of females, resulting in lower food requirements during the low prey density. However, even considerable decline in prey density in the third decade after the lynx reintroduction in Slovenia, simulated as a up to 40% decrease in fecundity, i.e. production of only one kitten that survives until end of its first year per female, did not lead to a population decrease.

The elasticity analysis revealed that adult survival is by far the most important demographic parameter for the lynx population dynamics. This is not surprising, considering the fact that lynx have a relatively long reproductive period (Stehlik 1984). Therefore, even minor decreases in survival rates could cause a decrease in population. Throughout history, carnivores have been perceived as man's competitors for prey and consequently directly persecuted (Sillero-Zubiri & Laurenson 2001). We presume that the controlled harvest of lynx as a trophy animal produced relatively positive attitudes of hunters toward the lynx in Slovenia and Croatia, which probably changed after its strict protection was put in place, and especially after the new hunting legislation has been adopted in Croatia.

Conclusions

Our 30–year simulations revealed that the reintroduced lynx population started declining when its survival rates were gradually decreased for 5%, simulating increased human related mortality in that period. Breitenmoser & al. (1999) believe that in Switzerland the expansion of lynx after reintroduction has ceased due to illegal killings. Even insignificant decreases in game densities or minor losses of livestock caused by lynx can make illegal killing the most important

mortality factor, as has probably happened in the case of the lynx in the Swiss Alps (Breitenmoser & al., 1998).

The hypothetical models of lynx population dynamics over a 100–year period highlighted two important factors that had a major impact on population growth dynamics and the related risk of population extinction: (1) changes in the survival rates of subadult and adult individuals in the population that are directly influenced by human activities and are difficult to control, and (2) the quality of habitat with regard to prey availability (density), which is relatively controllable parameter trough management of prey species in the lynx areal. The changes in the survival rate are by and large governed by the direct influence of man (legal hunting, illegal hunting, traffic mortality). Of these factors, legal hunting is the only one over that we can exert direct control or management. The other two factors, especially illegal hunting, can be affected only indirectly through long-term education and actions that decrease predator-caused conflicts.

Effects of habitat quality can have a major impact even when there is a minimal change in the survival rate of subadults and adults in the population. The most important factor affecting prey availability is the regulation of population size of prey species by man. Natural competitors for food, especially wolves (Jedrzejewski & al. 1993), also affect prey availability. Quite opposite to the adult survival parameter, the quality of habitat with regard to prey availability can be directly influenced through management. Since habitat quality can have a significant role for the lynx population dynamics and viability, in presence of even minor changes in survival rate of subadults and adults in a population, we must consider an adequate prey species management to be one of the most important short-term conservation priorities. Many values used for the PVA model are quite arbitrary, since there are no or few hard data for Dinaric lynx population and such an approach is the only choice. Therefore, the results should be used with caution, as some future simulations with new/better data may show different results.

Povzetek

Reintrodukcija oziroma ponovna naselitev osebkov določene vrste na območje, kjer je bila njena populacija v preteklosti iztrebljena (IUCN, 1995), je postala v zadnjih desetletjih ena izmed pomembnejših oblik upravljanja z ogroženimi živalskimi vrstami (Reading & Clark 1996, Wolf & al. 1996). Ponovno naselitev risa v Sloveniji leta 1973 lahko torej uvrščamo med pionirske naravovarstvene ukrepe, ki jih danes ciljno proučuje tudi varstvena biologija. Namen tega poglavja je predstaviti pomen posameznih demografskih parametrov ter njihov vpliv na populacijsko dinamiko in viabilnost dinarske populacije risa. Kljub relativno majhnemu številu populacijskih parametrov potrebnih za izdelavo osnovnih modelov populacijske dinamike je za dinarsko populacijo risa znanih le malo parametrov. Zato smo bili pri oblikovanju modelov prisiljeni uporabiti objavljene podatke o biologiji risa iz drugih evropskih populacij.

Za matematično modeliranje populacijske dinamike risa smo uporabili programski orodji RAMAS Metapop® (Applied Biomathematics, ZDA). Program omogoča oblikovanje in simulacijo (meta)populacijskih modelov s prostorsko strukturiranostjo. Vse analize in modeli so bili izdelani za dinarsko populacijo risa kot celoto na celotnem območju prisotnosti, saj populacijske dinamike in analize preživetvenih sposobnosti populacije ni mogoče obravnavati le za posamezen del populacije. Mehanizme, ki vplivajo na populacijsko dinamiko pri reintroduciranih populacijah risa delimo v dve fazi. V prvi fazi populacijske rasti je disperzija mladih osebkov pomemben dejavnik, ki omogoča visoko preživetje ter hitro prostorsko širjenje populacije. Gre za preprost matematični fenomen, ki ga dodatno ojači še naiven plen, ki nima izkušenj z novim plenilcem. Prva faza, hitro širjenje dinarske populacije risa, je najverjetneje potekalo do konca 80. oziroma začetka 90. let, ko se je upočasnilo oziroma ustavilo.

Po prenehanju prostorskega širjenja ter prilagoditvi plena na novega plenilca sledi druga faza populacijske dinamike, v kateri pride do zmanjšanje populacijske gostote oziroma ustavitve rasti populacije. V tej fazi je gostota plena, če niso prisotni drugi neposredni dejavniki, kot

so lov in krivolov, najpomembnejši dejavnik, ki vpliva predvsem na preživetje mladičev.

Deterministična stopnja rasti populacije je bila pri optimalni rasti lambda = 1,2, pri suboptimalni pa 1,15. To pomeni, da se v prvem primeru populacija vsako leto teoretično poveča za 20%, v drugem pa za 15%. Analizirali smo elastičnost posameznih demografskih parametrov (Tabela 1). Ugotovili smo, podobno kot Andren & al. (1998), da je največji vpliv na populacijsko dinamiko imelo preživetje odraslih osebkov, saj je spreminjanje parametra preživetja odraslih osebkov prispevalo kar 46–49% k spreminjanju populacijske dinamike populacije ter kvaliteta prostora z vidika dostopnosti (gostote) plena, ki neposredno vpliva na produkcijo mladičev (Tabela 1, Slika 2). Na spreminjanje stopnje preživetja ima največji vpliv človek s svojim neposrednim vplivom (zakoniti lov, promet, nezakoniti lov). Pri tem je le zakoniti lov dejavnik, ki ga lahko neposredno kontroliramo oziroma uravnavamo. Na ostala dva dejavnika, predvsem na nezakoniti lov, lahko vplivamo le posredno z dolgoročnim izobraževanjem in ukrepi, ki zmanjšujejo konfliktnost plenilca. Učinki kvalitete prostora so lahko izrednega pomena že pri minimalnem spreminjanju stopnje preživetja doraščajočih in odraslih živali v populaciji.

S simulacijami populacijske dinamike smo predstavili teoretična izhodišča razvoja dinarske populacije risa in njihove potencialne posledice v prihodnjih 100 letih. Za izhodišče modela smo uporabili začetno velikost populacije 100 osebkov s starostno in spolno strukturo, ki je bila blizu izračunani stabilni populacijski strukturi. Pri tem smo uporabili enake populacijske parametre kot v prvem delu s povprečno produkcijo 1,6 mladiča/samico. V model smo vključili Aleejev efekt, katerega učinki bi se pokazali šele v primeru, ko bi številčnost risa padla pod 20 osebkov v populaciji. Populacijo smo omejevali pri povprečni številčnosti K = 110 osebkov s stohastičnimi nihanji +–20 osebkov. Pri simulacijah populacijske dinamike in analize viabilnosti smo populacijo izpostavili dvema različnima situacijama v okolju. V prvem primeru smo simulirali populacijsko dinamiko risa v okolju z visoko gostoto plena (srnjadi), ki se kaže z visoko produkcijo mladičev (Okarma & al. 1997) (F = 1,6). V drugem primeru pa smo simulirali razmere v okolju z nizko gostoto plena v okolju, kjer smo v model vključili za 25% oziroma 40 % manjšo produkcijo oziroma preživetje mladičev. Za dane vrednosti smo ugotavljali učinke zmanjševanjanja stopnje preživetja risov (S), kot posledice dodatne smrtnosti, na populacijsko dinamiko in z njo povezana tveganja za izumrtje populacije v obdobju 100 let (Slika 3). V okolju z visoko gostoto plena (F = 1,6) se verjetnost izumrtja populacije risa pri zmanjšanju preživetja za do 10 odstotnih točk ni zmanjševala. Nasprotno pa se je verjetnost izumrtja naglo povečevala pri nadaljnem zmanjšanju preživetja risov kot posledice dodatne smrtnosti (Slika 3). Pri simulacijah populacijske dinamike v okolju z nižjimi gostotami plena (srnjadi) (F = 1,0 in 1,2) smo ugotovili, da bi se v okolju z nižjo gostoto plena številčnost populacije risa začela zmanjševati že pri zmanjšanju povprečne stopnje preživetja za 5 odstotnih točk, tveganje za njeno izumrtje v stoletnem obdobju pa bi se pri dodatnem zmanjševanju preživetja začelo hitro povečevati in bi pri zmanjšanju preživetja doraščajočih in odraslih osebkov za 15 odstotnih točk populacija zanesljivo izumrla v obdobju 45 let (Slika 3). Za razliko od parametra stopnje preživetja odraslih osebkov, lahko človek v veliki meri neposredno vpliva na parameter kvalitete prostora z vidika dostopnosti hrane. Zato je lahko primerno upravljanje s populacijami plenskih vrst eden izmed prioritetnih varstvenih ukrepov pri pripravi strategij upravljanja z dinarsko populacijo risa na celotnem območju njene razširjenosti.

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Using carbon fibre microelectrodes to monitor the oxidative metabolism of blowfly eyes

Uporaba mikroelektrod iz ogljikovih vlaken za spremljanje oksidativnega metabolizma mušjih oči

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Abstract. The oxidative metabolism in animal tissues can be conveniently monitored by measuring tissue P_{O2} with a carbon fibre microelectrode. We have established a recording configuration in a living animal by insertion of a carbon fibre electrode (CFE) into the retina of a blowfly (*Calliphora vicina* – chalky). The current flowing over an exposed carbon disc at the tip of an insulated carbon fibre with 5 µm diameter is linearly proportional to P_{O2} when the P_{O2} was varied between 0 kPa (100% N_2) and 100 kPa (100% O_2) in the recording chamber. The slight changes in sensitivity of CFE during the recording time were corrected by calibrations performed at the start and at the end of the experiments. Exposure of the eye to bright light caused a drop in tissue P_{O2}. Hypoxia increased with the stimulation time, reaching a maximum after about 20 s (ΔP_{O2}=11.6 kPa). These results are in good agreement with direct measurements of O_2 consumption in isolated eyes.

Keywords: blowfly eye, *Calliphora vicina* – chalky, carbon fibre electrode, P_{O2} measurement, amperometry

Izvleček. Oksidativni metabolizem živalskih tkiv je možno priročno spremljati s pomočjo meritev P_{O2} v tkivu z mikroelektrodami iz ogljikovih vlaken. Pri našem delu smo uporabili merilno konfiguracijo pri živi živali, tako da smo v retino muhe (*Calliphora vicina* – chalky) vstavili elektrodo iz ogljikovih vlaken (CFE). Tok, ki je tekel čez izpostavljen disk na konici izoliranega ogljikovega vlakna premera 5 µm, je bil premo sorazmeren P_{O2}, če smo P_{O2} spreminjali med 0 kPa (100% N_2) in 100 kPa (100% O_2) v merilni kamrici. Izpostavitev očesa močni svetlobi je povzročila padec P_{O2} v tkivu. Hipoksija se je povečevala s časom osvetlitve in je dosegla maksimum pri osvetlitvah dolgih približno 20 s. Ti rezultati se dobro skladajo z neposrednimi meritvami porabe kisika izoliranih oči.

Ključne besede. mušje oko, *Calliphora vicina* – chalky, ogljikova elektroda, merjenje P_{O2}, amperometrija

Introduction

The photoreceptors of animal eyes collect optical information of the environment, contained in the incident photon flux. The phototransduction process of the photoreceptors converts the incident light into a change in the photoreceptor's membrane potential, and this signal is subsequently transmitted to the animal's central nervous system. The phototransduction process requires an ionic imbalance across the photoreceptive plasma membrane and thus metabolic energy to power the ion pumps. The necessary power is provided by ATP, which is produced by the mitochondria. The mitochondrial activity of insect photoreceptors has been shown to be tightly coupled to the process of phototransduction (Tsacopoulos & al. 1983). Although this tight coupling, which in honeybee drones even precedes the actual changes in ion gradients, has been demonstrated a while ago, its nature and mechanism remains unknown. The most likely agent is the increase in $[Ca^{2+}]_i$, following the opening of the TRP and TRPL transduction ion channels.

The oxidative metabolism of insect eyes has been studied by various methods. The most direct approaches, where the actual oxygen consumption of the tissue is measured directly, require isolation of the eyes (Hamdorf & al. 1988, Pangršič & al. 2005). The eye needs to be put into a closed container, which prevents any other experimental manipulations like electrophysiological measurements. Other approaches include monitoring the redox states of the respiratory pigments (Tinbergen & Stavenga 1986, Mojet & al. 1991, Tinbergen & Stavenga 1987, Zupančič 2003) and monitoring the tissue P_{O2} (Tsacopoulos & Poitry 1982, Widmer & al. 1990, Poitry & Widmer 1996, Poitry & al. 1996) using polarization electrodes. In ideal circumstances, when the sample geometry is simple and the oxygen diffusion can be properly modelled, the latter method allows the transformation of the P_{O2} values into O_2 consumption. However, this can only be done with perfused slices of insect eyes, which again presents some limitations for other experimental procedures.

The aim of the present study was to record changes in P_{O2} in response to illumination of the blowfly eye *in situ*. For this we used carbon fibre polarization electrodes and amperometrically measured the P_{O2} within the retinal tissue.

Material and Methods

Experiments were done on male blowflies, *Calliphora vicina*, white-eyed mutant chalky. Adult flies were kept under a 12/12 h light-dark cycle and fed sucrose. Larvae were grown on liver, to assure a high rhodopsin content of the photoreceptors. We used flies between one and three weeks of age. Preparation was done under white light. The legs were removed and the animals were attached to a copper holder with a thin yoke around the neck of the animal in order to immobilize the head. The abdomen and mouth apparatus were glued to the holder using a 5:1 mix of bee wax and colophony. This mounting procedure allowed immobilizing the animal while keeping the tracheal openings unobstructed. The copper holder was placed at a cork support, attached to a microscope slide. The support also had a socket for attaching the reference electrode, made of a chlorinated silver wire. The reference electrode was manually inserted in the eye margin. For the insertion of the carbon fibre electrode we made a triangular incision in the same eye, thus removing ~10–20 facets from the cornea. The entire support, with the fly, was placed inside a plastic chamber, which allowed changes of the atmosphere surrounding the animal with a rapid gas-exchange system (Zupančič 2003). The chamber had a small window through which the carbon fibre electrode could be entered and subsequently inserted into the eye. The recording chamber was placed at the stage of a modified Leitz Orthoplan microscope, below the microscope objective (Leitz Plan Fl 4, 0.14 NA), so that the eye of the fly was in the focal plane of the objective (Fig. 1). The light beam of a 900 W xenon arc lamp (Osram, Germany) filtered by a blue interference filter (476 ± 10 nm; Schott, Germany) delivered the stimulus via the epi-illumination pathway. The final light intensity was adjusted using neutral density filters. Light was turned on and off by a mechanical shutter (Compur, Germany), controlled by the Spike 2 sequencer program (CED, Cambridge, UK) and a CED1401 plus (CED, Cambridge, UK) A/D

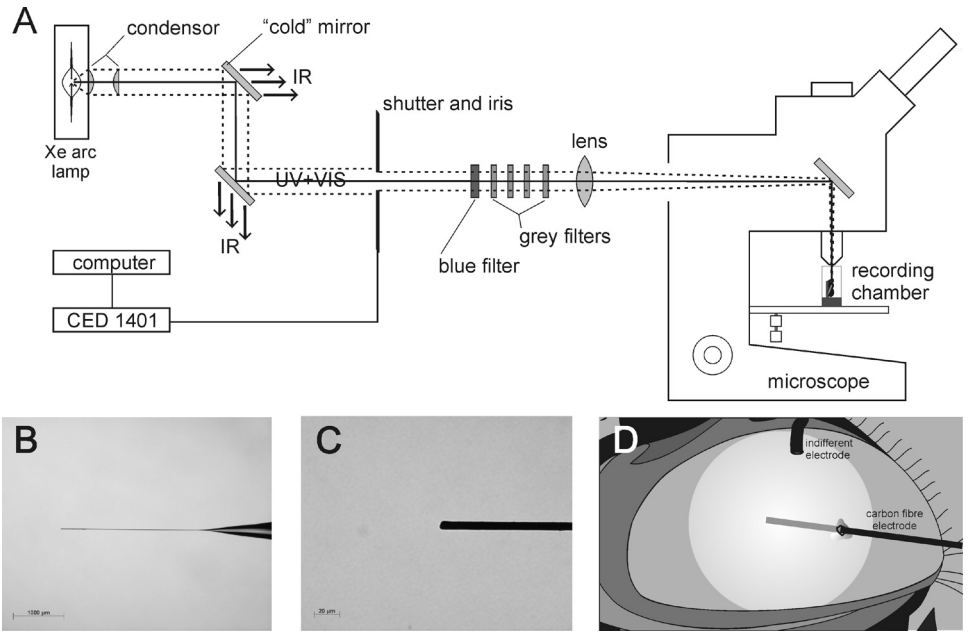

Fig. 1: **Diagram of the experimental apparatus, the preparation and the electrodes used. A –** All experiments were done on a modified Leitz Orthoplan microscope using a 900 W Xe arc lamp. Light was filtered with a 473 ± 10 nm blue filter. The recording chamber, placed under the microscope, allowed rapid gas exchange. **B** – We used electropainted, 5 µm diameter carbon fibre electrodes for measuring the P_{O2}. **C** – The insulated carbon fibre was cut with a scalpel blade to expose a disc-shaped, electroactive surface area. **D** – Positions of the carbon fibre and the reference Ag/AgCl electrode in the eye. The carbon fibre electrode was inserted through a pre-cut opening.

Slika 1: **Shema sistema za osvetljevanje, položaj ogljikove in referenčne elektrode v očesu in slika ogljikove elektrode. A** – vsi poskusi so bili izvedeni na modificiranem mikroskopu Leitz Orthoplan s pomočjo 900 W Xe obločne žarnice. Svetlobo smo filtrirali z modrim filtrom 473 ± 10 nm. Mehanski zaklop je vklapljal in izklapljal svetlobo. Merilno kamrico, ki je omogočala hitro zamenjavo plinov smo postavili pod mikroskop. **B** – Za meritve P_{O2} smo uporabili ogljikovo vlakno preseka 5 µm izolirano z elektrodepozitno barvo. **C** – Izolirano ogljikovo vlakno smo pred poskusom vsakič prerezali s pomočjo skalpela, da smo izpostavili diskasto aktivno površino. **D** – Poziciji ogljikove in referenčne Ag/AgCl elektrode v očesu. Ogljikovo elektrodo smo vstavili skozi poprej izrezano odprtino.

converter. The duration of the light stimuli was 0.03, 0.06, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 s. The interval between the light pulses was chosen manually to allow the response to each stimulus to decay back to the initial level before the next stimulus.

For amperometric P_{O2} measurements we used electropainted, 5 µm diameter carbon fibre electrodes. The electrodes were manufactured according to the procedure of Schulte and Chow (Schulte & Chow 1996, Chow & Rüden 1995). Briefly, a single carbon fibre was attached to the stripped end of a copper wire using silver conductive paste (Bison, Netherlands). The fibre was inserted into a borosilicate tubing, from which a microelectrode was then produced with a microelectrode puller. The gap between the glass tubing and the carbon fibre was sealed with silicone coating (Dow Corning Corporation, USA). The carbon fibre was isolated using anodic electrodeposition paint (Glassphor ZQ 84-3122; BASF, Germany) by applying a 2.5 V voltage for 3 min between the carbon fibre electrode and a platinum wire. Prior to each experiment, the tip of the elec-

trode was cut with a scalpel blade, thus exposing a disc-shaped electro-active surface area.

During recording the carbon fibre electrode was held at a polarisation voltage of –600 mV (relative to the reference electrode). The majority of current at this voltage is attributable to the reduction of oxygen (Mojet & al. 1997). The electrode current was measured with a home-made current to voltage converter feeding into a CyberAmp 380 (Axon Instruments, UK) amplifier. The signal was filtered below 6 Hz and sampled at 100 Hz.

Carbon fibre calibration procedure

In order to verify the basic assumption of linearity between the amperometric current and P_{O2} we tested the response properties of the carbon fibre electrode within the eye tissue. We examined this relationship using dead blowflies that were killed by hyperthermia (exposure to 50 °C for 2 min) to avoid problems due to the oxidative metabolism of active, live tissue (Fig. 2). We changed the P_{O2} in the recording chamber with a Cole-Parmer (USA) mixing flow meter in steps from 0 to 100 kPa and measured the resulting cur-

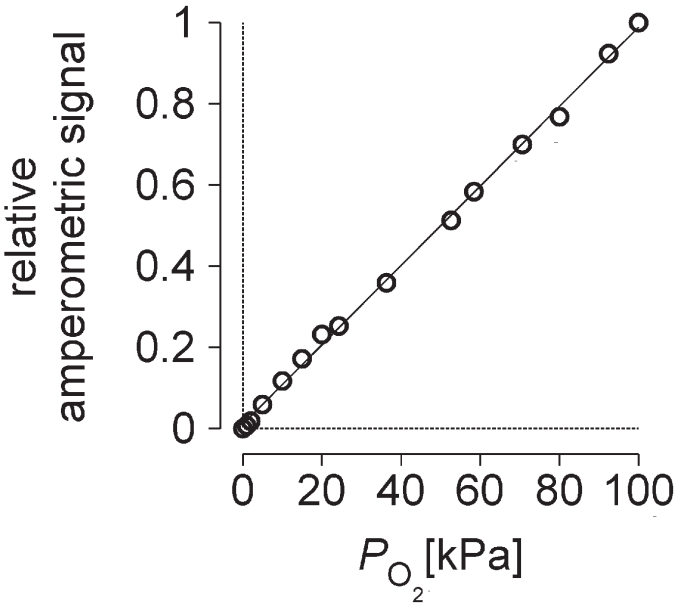

Fig. 2: **The relationship between P_{O2} and the relative amperometric signal (relative to maximal current in pure O_2) from the carbon fibre electrode.** Four blowflies killed by hyperthermia were exposed to different preset levels of environmental P_{O2} determined by a mixing flow-meter and verified with an electrochemical sensor. Since the sensitivity of four electrodes slightly differed, the values from each recording were normalized to the maximal current recorded at 100 kPa O_2. The ± 1 s.d. limits are in all cases smaller than the diameter of the circles indicating means. The relationship between the P_{O2} and relative amperometric signal was fitted with a linear function.

Slika 2: **Odvisnost med P_{O2} in relativnim amperometričnim signalom (relativnim glede na maksimalni tok v čistem O_2), izmerjenim z ogljikovo elektrodo.** Štiri muhe, usmrčene s hipertermijo, smo izpostavili različnim vrednostim P_{O2}, določenimi z mešalnim flow-metrom in preverjenimi z elektrokemičnim senzorjem. Ker se je občutljivost štirih uporabljenih elektrod nekoliko razlikovala, smo normalizirali vrednosti glede na makasimalni tok izmerjen pri 100 kPa O_2, ki je dal relativni amperometrični signal vrednosti 1. Meje ± 1 s.d. so v vseh primerih manjše od premera krogov, ki označujejo povprečja. Odvisnosti med P_{O2} in relativnim amperometričnim signalom je možno prilagoditi tudi linearno funkcijo.

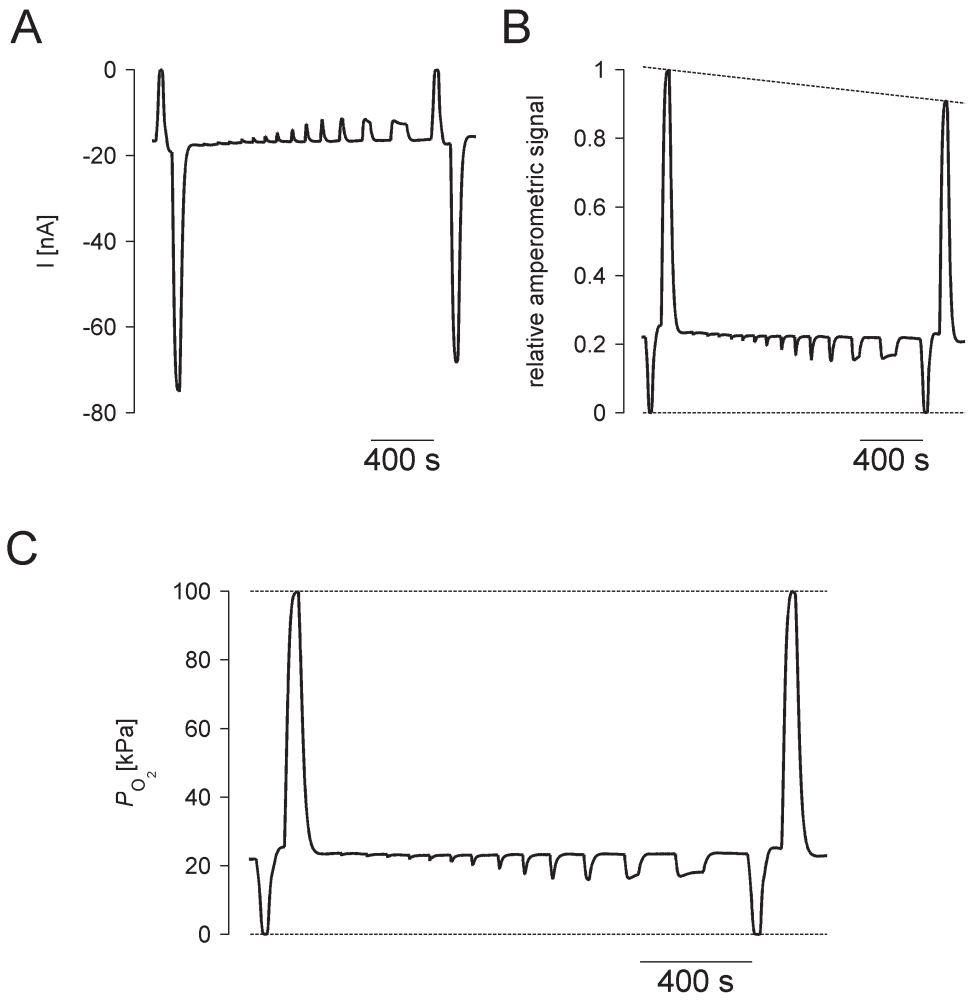

Fig. 3: **The calibration procedure and the correction for changes in sensitivity.** The measuring protocol consisted of: exposure to N_2 and O_2 calibrating pulses at the beginning, a series of illuminations, and a second exposure to N_2 and O_2 exposure at the end. The current in the electrode (**A**) was normalized with respect to the maximal current recorded in pure O_2 at the start of the recording (**B**). Because the electrode sensitivity changed during the experiment (**B**), a linear interpolation between the calibrating points in N_2 and O_2 was made, and the relative signal was transformed into P_{O2} values (**C**).

Slika 3: **Postopek kalibracije in korekcije zaradi sprememb v občutljivosti elektrode.** Merilni protokol so sestavljali izpostavitev kalibracijskim pulzom N_2 in O_2 na začetku, serija osvetlitev in druga izpostavitev N_2 in O_2 na koncu. Tok iz elektrode (**A**) smo normalizirali glede na maksimalni tok v čistem O_2 na začetku poskusa (**B**). Ker se občutljivost elektrode spreminja med poskusom (**B**), smo uporabili linearno interpolacijo med kalibracijskimi točkami v N_2 in O_2 in nato transformirali relativni signal v vrednoti P_{O2}. (**C**).

rent in the carbon fibre electrode. The P_{O2} values were verified independently with an electrochemical P_{O2} sensor (ECHO, Slovenia). Data from four blowflies are shown in Fig. 2. The sensitivity of electrodes used slightly varied, and therefore the values from each recording were normalized to the maximal current recorded at 100 kPa O_2 in order for the results to be comparable. The relationship between current and P_{O2} in the eye of a dead blowfly appeared to be linear, in accordance with Faraday's law describing the linear relationship of the number of reacting molecules and the total charge transferred. Having demonstrated the linearity of the carbon fibre electrode current with P_{O2}, we calibrated the electrodes prior to each experiment at two points: the signal at 0 kPa O_2 was given the value 0 (kPa) and that at 100 kPa O_2 obtained the value 100 (kPa). Intermediate values (>0 kPa and <100 kPa) were then calculated by linear interpolation (Fig. 3c).

The actual measurement protocol consisted of the following steps: at the beginning of an experiment, the preparation was exposed to pure N_2 and pure O_2, and subsequently a series of light pulses was applied; at the end of the protocol, the preparation was again exposed to pure N_2 and pure O_2. The last two calibration points allowed the correction for drift in the properties of the carbon fibre electrodes, which most likely are due to contamination of the active surface (Fig. 3b, c). The correction was made by linear interpolation between the calibration points before and after the experiment.

Results

The P_{O2} in the blowfly eye tissue drops upon illumination. The decrease in amplitude depends on stimulus duration and intensity (Fig. 4a). The largest decrease observed, with 20–50 s bright light illumination, was 11.6 kPa. On average, however, with intermediate light intensities (the photon flux averaged over the entire surface of the eye was 10^{16} photons s^{-1} m^{-2}) the decrease in P_{O2} with 20 s light pulses was 9.6 ± 0.7 kPa (mean

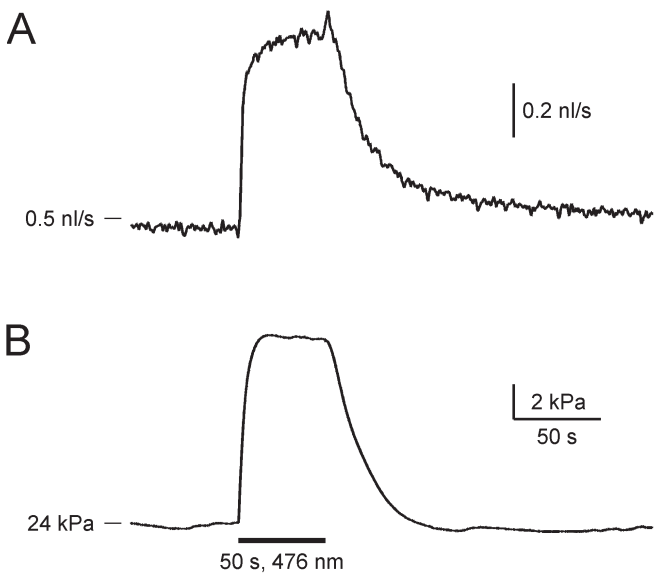

Fig. 4: Comparison of the time courses of the increase in O_2 consumption (A; adapted from Pangršič & al. 2005) and the absolute change in tissue P_{O2} (B).

Slika 4: Primerjava med časovnim potekom povečanja porabe O_2 (Pangršič & al. 2005) in časovni potek spremembe P_{O2} v tkivu.

± SEM, n=5). In such conditions the actual time course of the absolute change in P_{O2} was very similar to the increase in the O_2 consumption, elicited with comparable stimulation parameters, measured directly using a magnetic diver balance (Pangršič & al. 2005, Fig. 4b).

The results were similar with shorter light pulses, the only difference being that shorter light pulses produced smaller changes in P_{O2}. Fig. 5a shows an example of responses to a series of light pulses of different durations, from 30 ms to 50 s. Here it has to be noted that the measured drop in P_{O2} strongly depended on the position of the electrode within the eye, i.e. the depth of insertion and the position with respect to the centre of the illuminated area. In our case we achieved reproducible values with the electrode positioning in a series of experiments with intermediate light intensities comparable to the range between 10^{17} and 10^{18} photons s^{-1} m^{-2} as recorded with the magnetic diver balance (Pangršič & al. 2005). The comparison is only approximate since the entire eye was not uniformly illuminated, as was the case with the diver balance. In the present experiments the light flux in the centre of the illuminated area was quite different from that at the eye periphery. Nevertheless, another qualitative similarity with the measurements of O_2 consumption is the relationship between the stimulus duration and ΔP_{O2} (Fig. 5b). The relationship shown is very similar to the one recorded with direct respirometry. It covers three log units of stimulus durations and it saturates with durations longer than 20 s.

Discussion

Monitoring the mitochondrial activity within living tissue or cells is a prerequisite for any research dealing with questions concerning the role of mitochondria within active cells. Insect and especially blowfly eyes have in the past been the object of this kind of research using direct and indirect methods. Indirect approaches involved measurement of the absorption (Tinbergen & Stavenga 1986, Smits et al. 1995, Stavenga 1995, Zupančič 2003) or fluorescence (Stavenga & Tinbergen 1983, Tinbergen & Stavenga 1986, Tinbergen & Stavenga 1987, Mojet et al. 1991) of the mitochondrial respiratory pigments, but they suffer from the problem how to quantita-

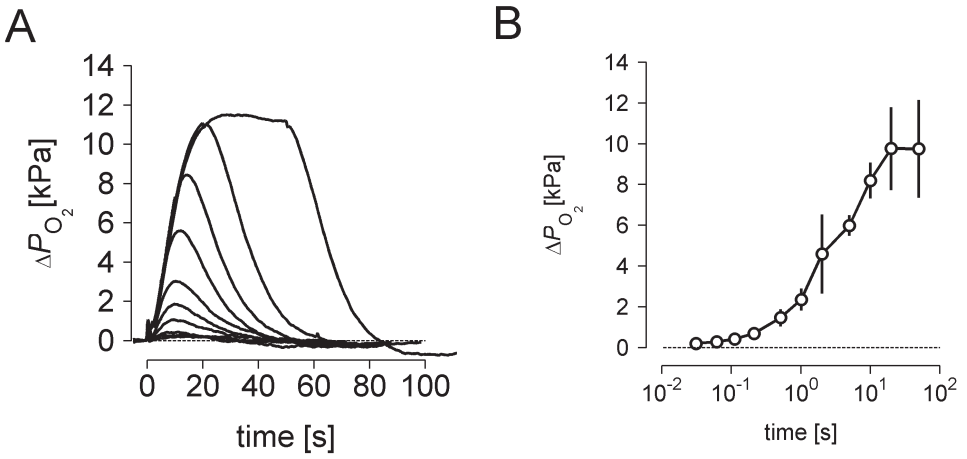

Fig. 5: **Responses to a series of light pulses of increasing duration. A –** Superimposed changes of P_{O2} in response to different stimulus duration. **B –** Dependence between light stimulus duration and changes in P_{O2}. Maximal changes are reached with 20 s light pulses.

Slika 5: **Odziv na serijo različno dolgih svetlobnih pulzov. A** – Superpozicija sprememb P_{O2} v odgovor na dražljaje različnega trajanja. **B** – Odvisnost med dolžino svetlobnih dražljajev in spremembami P_{O2}. Maksimalne spremembe so bile dosežene z 20 sekundnimi pulzi.

tively relate the measured parameters to the actual energy consumption. On the other hand, the direct measurement of the consumed O_2 (Hamdorf & al. 1988, Pangršič & al. 2005) is experimentally very demanding and in most cases prohibits any other manipulations of the preparation. We therefore have developed the measurement of the P_{O2} within the blowfly eye tissue with the carbon fibre electrodes, which are normally used for amperometric monitoring of secretion of oxidizable compounds (Mojet & al. 1997). We were able to successfully adapt the electrodes and the recording apparatus to measure the tissue P_{O2}, and we were able to at least qualitatively relate the recorded changes in P_{O2} to direct recordings of time courses of O_2 consumption using a magnetic diver balance (Pangršič & al. 2005). We found that both the time courses of the changes in P_{O2} as well as the relationship between stimulus duration and the consequent change in P_{O2} were comparable to the results obtained by direct measurements of the increase in O_2 consumption measured in isolated eyes.

Our main findings were that the carbon fibre electrodes are a good tool for measuring the P_{O2} within the tissue, providing some caveats are observed:

1. Small diameter carbon fibre electrodes of the type we used are prone to changes of their sensitivity when used within the tissue, either due to contamination of the surface area or to damage of their insulation. Often neither can be avoided, so a means of calibrating each recording before and after the experimental procedure must be assured. Our experimental animals, the flies, have a very high hypoxic tolerance, and also have a tracheal system that allows rapid exchange of gases deep within the tissue. It thus was a simple case of exposing the animal to pure N_2 and pure O_2 at the beginning and at the end of each experiment. Linear interpolation of the measured data between these two time points allowed adequate corrections for changes in sensitivity. This method is therefore very suitable for use in insects, provided they can tolerate an extremely low and extremely high P_{O2} for any length of time.
2. The carbon fibre electrode only records the P_{O2} locally. If the O_2 consumption varies within the tissue, the recorded values will reflect this. For the purpose of comparability, the recording sites therefore have to be as much standardized as possible.
3. Also it has to be noted that the P_{O2} represents the balance between the O_2 consumption and O_2 delivery. Normally we would like to correlate the P_{O2} to O_2 consumption, but this is only true when the rate of delivery does not change. However, even in insects this is not entirely true. Large loads on O_2 consumption are bound to trigger homeostatic mechanisms, which increase O_2 delivery, like opening of stigmata and ventilation movements, which will show up in the P_{O2} records. A possible example of this can be seen in figures 3 and 5, where P_{O2} actually increases with long illumination times of 50 or 60 s.

In conclusion, we have shown that carbon fibre electrodes can be used successfully to monitor P_{O2} within live tissue, especially in the eyes of flies and presumably also in other insects. The method has some limitations, which can easily be dealt with by proper design and execution of the experiments.

Povzetek

Fotoreceptorji posredujejo svetlobno informacijo iz okolja v živčni sistem živali. V tem procesu se svetlobna energija pretvori v električni odziv receptorske celice. Za proces fototransdukcije je nujno vzdrževanje ionskih gredientov prek celičnih membran, ki pa zahteva precej energije, zato morata procesa fototransdukcije in aktivacije mitohondrijev biti tesno povezana. Te procese so v preteklosti študirali pri žuželkah. Na tesno povezavo kaže starejše poročilo (Tsacopoulos & al. 1983), saj pride do povečanega delovanja mitohondrijev v retini čebeljih trotov pred spremembami ionskih gradientov, najverjetneje na račun povečanja $[Ca^{2+}]_i$ zaradi odprtja transdukcijskih ionskih kanalčkov TRP in TRPL. Najbolj natančne podatke o delovanju mitohondrijev v mušjih očeh so dale neposredne meritve porabe kisika (Hamdorf & al. 1988, Pangršič & al. 2005). Resna pomanjkljivost teh metod je, da potekajo na izoliranih očeh, zaprtih v drobno kamrico, ki preprečuje dostop za opravljanje dodatnih sočasnih meritev, na primer elektroretinografije. Vendar lahko energijski metabolizem spremljamo

tudi prek sprememb P_{O2} v tkivu. Uvedli smo merjenje P_{O2} prek izpostavljene površine 5 µm ogljikovega vlakna. Šlo je za amperometrične meritve, pri polarizacijski napetosti –600 mV, kjer večino večino toka prispeva redukcija kisika. Elektrode z majhnim presekom pa imajo pomanjkljivost – njihova občutljivost se tekom poskusa spreminja, bodisi zaradi kontaminacije aktivne površine ali zaradi poškodb izolacije. Umeritev elektrode pred vsakim poskusom je torej nujna. Žuželke, in predvsem muhe, imajo kot poskusne živali v tem primeru dve veliki prednosti. Trahealni sistem omogoča hitro izmenjavo plinov globoko v tkivu, živali pa so izjemno odporne na anoksijo. Umeritve smo izvedli tako, da smo pred in po vsakem poskusu izpostavili živali čistemu O_2 in čistemu N_2. Z linearno interpolacijo med temi umeritvenimi točkami smo korigirali spreminjanje občutljivosti elektrod. Upoštevati moramo tudi, da z ogljikovimi elektrodami merimo spremembe P_{O2} zelo lokalno. Če poraba O_2 v tkivu ni enakomerna, bo to odsevalo tudi pri meritvah P_{O2}. Položaj elektrode mora biti zato kar najbolj standardiziran. P_{O2} v tkivu in poraba O_2 sta povezana, vseeno pa ju ne gre povsem enačiti. P_{O2} namreč predstavlja ravnotežje med porabo in dostavo O_2. Veliko metabolno breme tako povroči še druge homeostatske mehanizme, ki povečajo dostavo O_2, kot so povečana frekvenca dihalnih gibov in odprtje stigem. Kljub temu pa so časovni poteki sprememb P_{O2} v tkivu ob osvetlitvi zelo podobni časovnim potekom sprememb porabe O_2 (Pangršič & al. 2005).

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Preferences for different substrates in *Phalangium opilio* (Opiliones: Phalangiidae) in natural environment

Preference navadnega matije, *Phalangium opilio* (Opiliones: Phalangiidae) do različnih substratov v naravnem okolju

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Abstract. *Phalangium opilio* is the most widespread and one of the most common harvestman species in anthropogenic environments. A preliminary field experiment was carried out in Slovenia testing its preferences for different substrates. A two metres high rectangular tower with walls constituted of vertical bands of concrete, twice fired tile, wood and styrofoam was placed in a meadow. The wood proved to be the most suitable substrate, providing the most stable temperatures and moisture levels in comparison with the other experimental materials. In anthropogenic environment, various available substrates in microhabitats of *Ph. opilio* considerably contribute to a fine regulation of searching relatively thermally and moist-stable resting sites.

Key words: harvestman, arachnids, Arachnida, Slovenia, substrate preference, synanthropy

Izvleček. *Phalangium opilio* je v svetu najbolj razširjena in najbolj običajna vrsta suhih južin v antropogenem okolju. Avtorji so preliminarno preizkušali preference vrste do različnih podlag v antropogenem okolju. Na travniku so postavili 2 m visok pravokoten preizkuševalni stolp iz stranic, sestoječih iz navpičnih pasov betona, dvakrat žgane opeke, lesa in stiropora. Les se je izkazal za najugodnejši substrat, ki zagotavlja najbolj stabilno temperaturo in vlago v primerjavi z ostalimi preskusnimi materiali. V antropogenem okolju omogočajo podlage iz različnih materialov izbor toplotno in vlažnostno najugodnejših mest za mirovanje.

Ključne beside: pajkovci, preferenca do substrata, sinanthropija, Slovenija, suhe južine

Introduction

Phalangium opilio Linnaeus, 1758 is the most widespread opilionid species in the World, living in Europe, North and Central Asia and Asia Minor; it was also introduced from Europe to North America, North Africa and New Zealand (Martens 1978, Edgar 1980, Blick & Komposch 2004). It inhabits open habitats, as meadows and mires/bogs, open/lightful forests, and various types of anthropogenic habitats, as gardens, agroecosystems, forest edges, hedgerows, lawns, quarries, and in urban centres green habitats, walls and bridges made of various materials (Todd 1949, Edgar & Yuan 1965, 1968, Clingenpeel & Edgar 1966, Martens 1978, Edgar 1980, Komposch 1993, Newton & Yeargan 2002, Novak & al. 2002, Hillyard 2005). It has been often discussed

as a synanthropic harvestman (Edgar & Yuan 1965, 1968, Martens 1978, Kuschka 1991, Komposch 1993, Thaler & Knoflach 1995, Klimeš 1997, Novak & al. 2002, Komposch & Gruber 2004, Merfield & al. 2004, Allard & Yeargan 2005, Willemart & al. 2006). In fact, the species belongs to the so called »Kulturfolger« sensu Povolný (1963) – meaning an organism occurring in cultivated landscapes (»following« such habitats) for ecological reasons, like coprophagous arthropods which are common in cattle herds and sheep flocks. In open sites with tall objects, *Ph. opilio* also climbs such objects. During bad weather it takes shelter in grass and the understory, as well as in anthropogenic habitats also beside different commercial, especially building materials.

In Central Europe, *Ph. opilio* matures stenochronously once a year in late summer and early autumn (Kaestner 1926, Rüffer 1966, Martens 1978), while there are more generations in North America (Edgar & Yuan 1968). The eggs and juveniles develop most rapidly at temperatures between 20−30°C, whereas their embryonic development is completely retarded at temperatures below about 10°C (Juberthie 1964, Rüffer 1966, Bachmann & Schäfer 1983). *Ph. opilio* is predominantly nocturnal, executing ca. 90% of its activity between 6 p.m. and 6 a.m. (Edgar & Yuan 1965, 1968). In natural habitats it is exposed to sun and wind, thus hardy in tolerance of desiccation and adapted to relatively high illumination levels (Edgar & Yuan 1968, Edgar 1980, Clingenpeel & Edgar 1966). In urban areas in Slovenia, petrophily has been found to be predominant in synanthropic harvestmen, including *Ph. opilio* (Novak & al. 2002).

Experimentally gathered data about the thermo- and hygropreference of *Ph. opilio* in the USA (Edgar & Yuan 1965, 1968, Clingenpeel & Edgar 1966) differ a lot from the field data recorded in the UK (Todd 1949). In Europe, these preferences have not been studied experimentally. Since the 1990s, the traditional areas for behavioural study have largely been abandoned, but many long-standing challenges remain (Owens 2006). The scope of this study was preliminary testing preferences of *Ph. opilio* for different substrates, and to find reasons for unequal affinities for different commercial, especially building substrates in human settlements. We also tried to contribute to an ecophysiological explanation for why *Ph. opilio* is a common species in human habitats.

Material and Methods

Phalangium opilio specimens: 37 ♂, 31 ♀ and three subadults used in the experiment were collected in Žavcarjev vrh in north-eastern Slovenia (46°36' N, 15°32' E, alt. 800 m). The experiment was arranged in a plane meadow near Slovenske Konjice in north-eastern Slovenia (46°21' N, 15°27' E, alt. 360 m) and carried out from August10th till September 18th 2005 when it was ended entirely because of cold, rainy weather.

In order to test preferences for different substrates, a square experimental tower was constructed with walls measuring 44 x 200 cm (Fig. 1). Each wall consisted of vertical belts – 1 cm thick and 11 cm wide – of the following four experimental surfaces, affixed to a wooden plate. Their thermal conductivity coefficient, λ, in W/mK, according to Engineering Toolbox (2006), is in parentheses: concrete (1.16), twice fired tile (1.05), pinewood (0.14) and styrofoam (0.04). The concrete and the wood were porous, while the tile, and the styrofoam due to its waterproof surface nonporous materials. These materials served as the arbitrary resting substrates for the specimens. The top of the tower was covered with a translucent plastic plate extending 10 cm over the edges, to avoid concealment of individuals inside. A 50 cm high plastic fence was erected 1 m from the tower to prevent escapes, and to provide a grassy habitat for hiding and hunting. Twice a week, meadow insects were caught and released inside the fence to provide the prey species availability by chance and choice. The experimental individuals were placed into the arena 10 days before starting the experiment to acclimate. The records were kept exclusively in nice days, and at least two days after a rainfall.

The measurements started at 1:00 p.m. to guarantee data from the hottest period of the day when *Ph. opilio* does not move (Edgar 1980). At the resting site of each specimen on the tower, the compass direction of the chosen wall, the height, the selected substrate, the air temperature (T) and the relative humidity (RH) 1 cm from the specimen's body, using a handheld aspiration psychrometer (Ahorn FN 864, Germany), were

Fig. 1: The experimental tower in a provisory site (the experimental meadow in behind).

recorded. The only records kept were of those specimens with the whole body and more than half of the legs positioned on a selected substrate. It was hypothesized, that during hot days 1) hotter the day, higher the settled site on the tower, and 2) stony surfaces (concrete, tile) are preferable to *Ph. opilio*.

For statistical purposes one way ANOVA was used in testing the differences between substrate in means for height, T and RH of the resting specimens. For testing differences in preferences, the Chi-square test was used. To provide the F test for RH, the data were *arcsin*-transformed. SPSS 12.01 for Windows was used in the procedures.

Results

During the experiment, the weather was good with two rainy intervals – each in a few days in duration. The number of specimens settled on the tower decreased logarithmically from on the first to the 37th experimental day (Fig. 2). Cumulatively, 90 measurements were provided, thus some records referred to the same specimens. During the time of the experiment, the subadults moulted. In their resting sites, there were no statistical differences between sexes and between adults and subadults in height, temperature (T) and relative humidity (RH) (One way ANOVA, $P > 0.05$); therefore the data for further statistical analyses were pooled. Individuals usually terminated their activity period by performing an exploratory movement for a few seconds within a radius of 10 to 25 cm before settling. On rainy days, they took shelter in the grass.

Near the surface of the experimental substrates, concrete was found to provide the least thermal stability (CV=18.4 %), and styrofoam the least humidity stability (CV=18.7 %), while wood was generally the most stable material with respect to both measurements (Table 1).

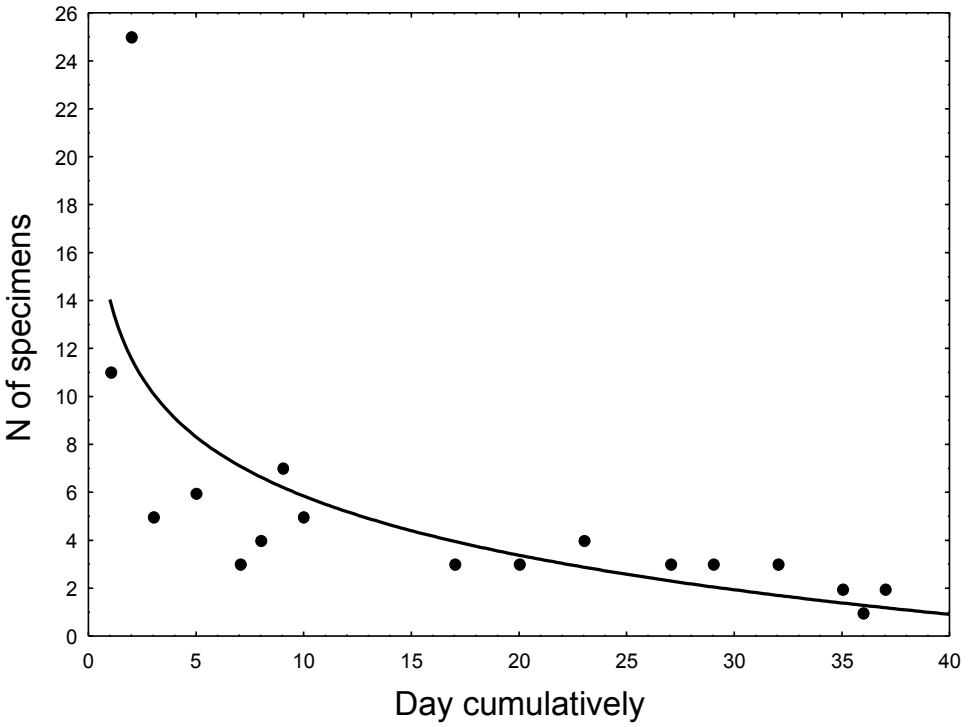

Fig. 2: Individual records of *Phalangium opilio* on the experimental tower diminished logarithmically during the experiment.

During their daytime rest, the specimens positioned themselves irregularly according to substrates (Table 1). The concrete, tile and styrofoam were comparable, while the wood was significantly more frequently occupied. Most specimens placed themselves on the wood, while the tile was the least preferred substrate ($\chi^2 = 11.96$, df = 3, p = 0.008).

The specimens spread over the whole range of the tower's height, in the range of mean heights between 43 and 160 cm, both extremes on the tile. Regardless of the compass direction, the T means for different substrates were between 20.9 and 24.9°C, both extremes on the concrete, and the RH mean between 58.1 and 79.0%, both on the styrofoam.

T and RH were in a moderate negative correlation with each other ($r = –0.73$, $P < 0.001$). On the one hand, the concrete, tile and styrofoam, which differ in their thermal conductivity 29-times (Engineering Toolbox 2006) were comparably frequently settled, while wood and styrofoam, on the other hand, which differ only 3.5-times, were not.

Discussion

Although harvestmen behave quite similarly in the laboratory as in natural habitats (Edgar & Yuan 1968), various unnatural circumstances in the laboratory, such as inappropriate experimental containers, light conditions, food etc., can influence the experimental results. In experiments with predators, the use of factitious prey can hardly be avoided, which cannot be favourable to the experimental specimens (Merfield & al., 2004). On the other hand, in nature, a range of instabilities often renders the interpretation of results difficult. The experimental tower in the meadow enabled

Table 1: Mean, standard deviation, minimum, maximum and coefficient of variation, CV, of the height, temperature, T, and relative air humidity, RH, at the sites of *Phalangium opilio* with respect to the substrates.

Substrate	N	Height (cm)	T (°C)	RH (%)
		Mean ± SD Min – Max CV (%)		
Concrete	17	55.0 ± 49.6 10 – 200 90.2	21.5 ± 4.0 16.1 – 27.1 18.4	73.6 ± 12.9 54.1 – 91.2 17.5
Tile	15	80.8 ± 60.8 10 – 200 56.5	23.0 ± 4.0 15.5 – 28.4 17.2	65.4 ± 10.4 47.4 – 88.1 16.0
Wood	36	80.8 ± 63.0 2 – 195 77.9	22.6 ± 3.2 15.7 – 29.1 14.3	66.2 ± 10.4 50.9 – 86.1 15.6
Styrofoam	22	81.9 ± 62.3 5 – 200 76.5	21.8 ± 3.1 13.9 – 26.8 14.1	67.0 ± 12.5 53.1 – 98.3 18.7

accurate measurements and optimally reflected responses of *Ph. opilio* within mixed artificial-natural habitats in the temperate zone.

In comparison with the T of 15.5°C found to be the thermopreference in field populations in Great Britain (Todd, 1949), and 27.6°C in an experimental one in the USA (Edgar, 1980), the mean preferred T of 17.8–23.2°C in our experimental population indicates that the preferences depend on thermal acclimatization in concrete habitats. In hot days, in *Ph. opilio*, adapted to habitats that are well sun-radiated (Edgar, 1980), the substrate selection among others helps in maintaining their thermal stability at relatively high levels in avoiding of hyperthermy at the same time.

The micrometeorological conditions for different substrates were strongly influenced by their properties. The existence of comparably frequently settled substrates that differ greatly in their thermal conductivity, on the one hand, together with unequally settled substrates that differ less, on the other hand, indicates that the thermal conductivity of a substrate does not by itself have an impact on the substrate selection. The concrete was thermally the least stable substrate because of its porosity, and different intensities of evaporation and desiccation during the day. The properties of tile were comparable. In spite of its low thermal conductivity, and although kept moist in the laboratory (Klee & Butcher 1968, Edgar 1980), in the field styrofoam displayed low moisture capacity. The preference for wood in *Ph. opilio* can be understood by its thermal stability as the consequence of its low thermal conductivity and the steady moderate release of moisture, both providing comparatively the most stable microclimate at a site. This is in agreement with the statements of Kuschka (1991), that *Ph. opilio* is a thermophilous and moderately hygrophilous species.

The persistence of *Ph. opilio* in the human settlements is the consequence of a combination of facts. In this opportunistic feeder (Martens 1978, Morse 2001), its thermophily and hardiness against desiccation and drought (Edgar & Yuan 1968, Edgar 1980) assure relatively fast metabolism and development. Individual selection of various substrates proved to be one of the T and RH regulation mechanisms, while the selection of compass direction, the height of climbing tall objects, as well as a cryptic behaviour during unpleasant weather should be tested with this respect in avoiding auto-replicates as further means of assuring relatively fine regulation of the optimal metabolism and development. In anthropogenic environment, various substrates available in microhabitats of *Ph. opilio* considerably contribute to a fine regulation of searching relatively thermally and moist-stable resting sites.

Conclusions

In conclusion,

- among commercial, especially building substrates, a wood is the preferable substrate to *Ph. opilio*,
- the apparent preference for concrete substrate in human settlements is the consequence of an irregular distribution of different substrates within the settlements.

Povzetek

Phalangium opilio je najbolj razširjena vrsta suhih južin in obenem najbolj običajna v antropogenem okolju. Pogosto jo obravnavajo kot sinantropno vrsto v širšem smislu. Preliminarno smo ugotavljali preference vrste do različnih podlag, na katerih osebki mirujejo v antropogenem okolju, ker takšni poskusi niso bili opravljeni. Stranice 2 m visokega preizkuševalnega stolpa so bile iz navpičnih pasov betona, dvakrat žgane opeke (klinkerja), smrekovega lesa in stiropora. Stolp je bil ograjen z 0,5 m visoko plastično ograjo, da poskusne živali niso ušle. Meritve temperature in vlage smo opravili tik ob osebkih po njihovi umiritvi od 13^{h} naprej, ko je bila temperatura najvišja. Les se je med preskusnimi materiali izkazal za preferenčen substrat, ker zagotavlja najbolj stabilno temperaturo in vlago. Poleg ostalih dejavnikov, kot so stran neba, višina namestitve nad tlemi in drugi, omogočajo različni materiali v antropogenem okolju osebkom suhih južin izbor toplotno in vlažnostno najugodnejše podlage za mirovanje.

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First report of cyanobacterial bloom of *Microcystis viridis* (A. Braun) Lemmermann in Slovenia

Prvi opis cvetenja cianobakterije *Microcystis viridis* (A. Braun) Lemmermann v Sloveniji

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Abstract. The presence of the cyanobacterial bloom of *Microcystis viridis* (A. Braun) Lemmermann is reported for the first time in Slovenia. After field sampling, and detailed microscopic observations, species analysis, chlorophyll content analysis, and cyanobacterial cyclic peptides were determined, the latter by high performance liquid chromatography (HPLC). Cells were found in colonies with limited amounts of more or less refractive mucilage. The average diameter of a cell was 4–7 µm. Three microcystins, two anabaenopeptins and planktopeptin BL 1125, were identified. The content of cyclic peptides in the bloom was in the range of 2.3–6.6 mg g^{-1} of cellular dry weight. *M. viridis* was dominant in the cyanobacterial bloom, other species being *Micorcystis wesenbergii, Microcystis aeruginosa, Anabaena flos-aque, Anabaena spiralis, Aulacoseira granulata, Closterium* sp.*, Euglena* sp.*, Pediastrum duplex, Scenedesmus quadricauda, Staurastrum gracile, Trachelomonas volvocina, Trachelomonas hispida* and *Tetraedron limneticum*. In keeping with previous studies the content of cyclic peptides in the cyanobacterial bloom was high enough to cause bloom lysis. This fact was also confirmed by field observation; not only bloom composition change, but after 8 days there was no visible cyanobacterial bloom on the Boreci reservoir surface, although no heavy rain or wind was observed during this period. The discovery of *M. viridis* bloom in Slovenia is very important, since toxic bloom constitutes a threat all over the World.

Key words. cyanobacteria, cyanobacterial bloom, *Microcystis viridis*, microcystin, cyclic peptides

Izvleček. V Sloveniji prvič poročamo o prisotnosti cianobakterijskega cveta *Microcystis viridis* (A. Braun) Lemmermann. Po vzorčenju, temeljitem mikroskopskem pregledu, analizi vrstnega sestava in analizi vsebnosti klorofila smo določili prisotnost cianobakterijskih cikličnih peptidov s pomočjo tekočinske kromatografije visoke ločljivostji (HPLC). Celice so v kolonijah obdane z sluzjo, ki lomi svetlobne žarke. Povprečni premer celic je bil 4–7 µm. Identificirali smo tri mikrocistine, dva anabaenopeptina in planktopeptin BL 1125. Vsebnost cikličnih peptidov v cvetu je bila 2.3–6.6 mg g^{-1} suhe celične mase. V cianobakterijskem cvetu je prevladovala vrsta *M. viridis*, ostale vrste pa so bile *Micorcystis wesenbergii, Microcystis aeruginosa, Anabaena flos-aque, Anabaena spiralis, Aulacoseira granulata, Closterium* sp.*, Euglena* sp.*, Pediastrum duplex, Scenedesmus quadricauda, Staurastrum gracile, Trachelomonas volvocina, Trachelomonas hispida* in *Tetraedron limneticum.* Na podlagi rezultatov prejšnjih raziskav lahko zaključimo, da je vsebnost cikličnih peptidov dovolj visoka, da lahko povzroči lizo cveta. To dejstvo je bilo potrjeno tudi z opazovanji v naravnem okolju v okviru te raziskave. Ni prišlo samo do spremembe

vrstne sestave cveta, temveč do izginotja cianobakterijskega cveta na površini jezera Boreci po osmih dneh, čeprav v tem obdobju ni bilo močnega deževja, niti vetra. Odkritje pojavljanja cvetenja *M. viridis* v Sloveniji je izrednega pomena, saj strupen cianobakterijski cvet predstavlja grožnjo po celem svetu.

Ključne besede. cianobakterije, cianobakterijski cvet, *Microcystis viridis*, mikrocistin, ciklični peptidi

Introduction

About 60 % of cyanobacterial samples investigated worldwide contain toxins (Guidelines 2003). The toxicity of a single bloom can, however, change in both time and space. Demonstration of toxicity of the cyanobacterial population in a given lake or reservoir does not necessarily imply an environmental or human hazard as long as the cells remain thinly dispersed. Mass developments and, especially, surface scums pose the major risk.

Microcystis has been known to be the major genus among the cyanobacteria to cause blooms in fresh waters worldwide (Carmichael 1992; Guidelines 2003). *Microcystis* blooms frequently occur in the eutrophic waters. In many north-eastern Slovenian lakes and reservoirs, nutrient loading, coupled with year-round warm weather, favours the growth of cyanobacteria, several of which can produce cyanotoxins, especially the potent genotoxins (Žegura & al. 2003) and liver toxins called microcystins (MC). The toxins are of interest due to their threat to humans and animals (Carmichael 1994; Falconer & al. 1994, 1999).

M. viridis has been found in Finnish fresh and coastal waters (e.g. Sivonen & al. 1990), a Swedish lake (Cronberg & al. 1999), a Brazilian reservoir (Figueredo & Giani 2001), China (Song & al. 1998) and Japan (Kameyama & al. 2004). Based on 16S rRNA analyses of *M. viridis* (Lepre & al. 2000) and DNA-DNA homology analysis (Kondo & al. 2000), some authors (Otsuka & al. 2001), under the rules of the bacteriological code, propose the unification of five species of

Table 1: Isolated peptides from cyanobacteria ***Microcystis viridis***.

***Microcystis viridis* strain**	**isolated peptides**	**reference**
M. viridis	cyanoviridin-RR	Kusumi et al., 1987
M. viridis NIES-102	cyanoviridin-RR	Ooi et al., 1989
M. viridis	microcystin-RR, -YR, -LR	Watanabe et al., 1989
M. viridis NIES-102	mikroviridin	Ishitsuka et al., 1990
M. viridis - axenic	microcystin-RR, -YR, -LR, -LA	Kaya & Watanabe, 1990
M. viridis – from bloom	microcystin-RR, -LR	Kaya & Watanabe, 1990
M. viridis NIES-102	hepatotoxic polypeptides	Yusamo & Sugaya, 1991
M. viridis NIES-102	aeruginosin 102-A, 102-B	Matsuda et al., 1996
M. viridis NIES-103	micropeptin 103	Murakami et al., 1997
M. viridis NIES-103	aeruginosin 103-A	Kodani et al., 1998
M. viridis– from bloom	microcystin-RR	Song et al., 1998
M. viridis NIES-102	polypeptide MVL	Yamaguchi et al., 1999
M. viridis NIES-102	microcystin-RR, -YR, -LR	Kameyama et al., 2002, 2004
M. viridis FACHB	cyclic peptides with *mcyB* gene	Pan et al., 2002

cyanobacterial genus *Microcystis: M. aeruginosa, M. ichthyoblabe, M novacekii, M. viridis* and *M. wesenbergii*. It has been recommended that attention should be paid to the occurrence and possibility of toxic blooms of *M. viridis* from the standpoint of water management and public health (Watanabe & al. 1986).

Peptides from *M. viridis* do not differ completely from other peptides from genus *Microcystis* (Table 1). Some *M. viridis* peptides are known to be active as essential intracellular nitrogen compounds in toxic cyanobacteria, substances active against grazing zooplankton (Yasuno & Sugaya 1991), a chymotrypsin inhibitor (Murakami & al. 1997) and as a mannan-binding lectin important for haemagglutination (Yamaguchi & al. 1999). Nevertheless, not much more is known about peptides from *M. viridis* and there is no report to date of *M. viridis* bloom occurrence in Slovenia.

Material and methods

Field sampling

Three sampling points were located in the north-eastern part of Slovenia. Gauss Krüger coordinates for reservoir Boreci (Križevci village) are y = 588239.7; x = 158373,3; z = 182m. For reservoir Podgrad (Podgrad village) y = 574341.4; x = 171416; z = 208m. For reservoir Hotinja vas (Hotinja village) y = 552400.4; x = 147283.2; z = 262m. The majority of results presented in this article are from reservoir Boreci, since the most extensive analyses were performed there. Samples were collected with the planktonic net, separately from the whole water column and surface scum.

Cyanobacterial and algal species

Species were identified using an inverted microscope according to Komarek (1991, 1999–2000), Starmach (1966) and Hindak (1978). The abundance on August the 9th was estimated with several dilutions of original sample and counting with haemocytometer. Samples from other dates did not show dominance of one, but four species, and the abundance with counting could not be estimated precisely enough (symbol + in Table 2). Samples were analysed for composition of plankton species and taxonomic determination under an inverted microscope (Nikon Eclipse TE300). Cells were measured with Lucia (System for Image Processing and Analysis LUCIA 4.6, Laboratory Imaging Ltd.).

Chlorophyll content analysis

Chlorophyll *a* was measured by methanol extraction according to Vollenweider (1969) with a spectrophotometer UV-2101 PC (Shimadzu). The procedure was modified to filtration of 10 ml samples in triplicate.

Cyanobacterial cyclic peptide analysis

The lyophilised bloom material was processed according to Harada & al. (1988) with minor modifications. Dried cyanobacteria (1000 mg) were extracted three times with 5% aqueous acetic acid (3 x 20 ml) for 30 min with stirring. The extracts were centrifuged at 4000 rpm for 10 min. The combined supernatants were applied to preconditioned 500 mg reversed-phase disposable columns (LiChrolut RP-18, Merck). The columns containing the extract were washed with 20 ml of 10 % methanol and the cyclic peptides eluted with 2 ml methanol (LiChrosolv, Merck), evaporated to dryness under nitrogen stream and the residues dissolved in 0.05 M phosphate buffer, pH 3. Samples were analysed by HPLC, using isocratic elution with methanol: phosphate buffer 48:52 (v/v). The HPLC/PDA equipment consisted of a Waters 600 Controller, Waters 616 pump and Waters PDA Detector. Millenium^{32} software (Ver. 3.0, Waters) was used to run the hardware and to process the data.

Identification and visualization of cyclic peptides with a photodiode array detector

The chromatogram was monitored at four wavelength maxima – 238, 225, 220 and 215 nm – in order to locate and distinguish MC from other bioactive cyclic peptides of interest. The wavelengths are characteristic of individual cyclic peptides; MCs have a characteristic absorption at 238 nm, while other isolated cyclic peptides have absorption maxima at lower wavelengths. The depsipeptide planktopeptin BL1125 was detected at 225 nm and anabaenopeptins B and F

Table 2: Bloom sample structure and dominant species (***present in footnote***) on two days in August 2006 from Boreci reservoir.

Bloom sample structure 9. 8. 2006	**Bloom sample structure 17. 8. 2006**
+ *Microcystis viridis 94 %*	+ *Microcystis viridis*
Microcystis wesenbergii 4%	+ *Microcystis wesenbergii*
Microcystis aeruginosa	+ *Microcystis aeruginosa*
Anabaena flos-aque	+ *Anabaena spiroides*
Anabaena spiralis	*Aphanizomenon flos-aque*
Aulacoseira granulata	*Aulacoseira granulata*
Closterium sp.	*Anabaena solitaria*
Euglena sp.	*Woronichinia naegeliana*
Pediastrum duplex	*Dictyosphaerium pulchellum*
Scenedesmus quadricauda	*Euglena* sp.
Staurastum gracile	*Pediastrum duplex*
Trachelomonas volvocina	*Staurastum gracile*
Trachelomonas hispida	*Trachelomonas volvocina*
Tetraedron limneticum	*Trachelomonas hispida*

+ = dominating species

at 215 nm. Both types of non-toxic cyclic peptide have additional characteristic absorption maxima at 278–279 nm that were used to confirm the preliminary identification (Grach-Pogrebinsky & al. 2003). The amounts of the cyclic peptides were calculated from the individual peaks by compari-

Fig. 1: Cyanobacterial bloom in Boreci reservoir, located in Križevci village, 9. 8. 2006, photo: Tina Eleršek.

son of the integrated peak areas with the values from calibration curves standardized by previously isolated cyclic peptides in pure form.

Results and discussion

This is the first report of cyanobacterial bloom of *Microcystis viridis* (A. Braun) Lemmermann in Slovenia. Microscopic examination of the phytoplankton samples showed the dominance

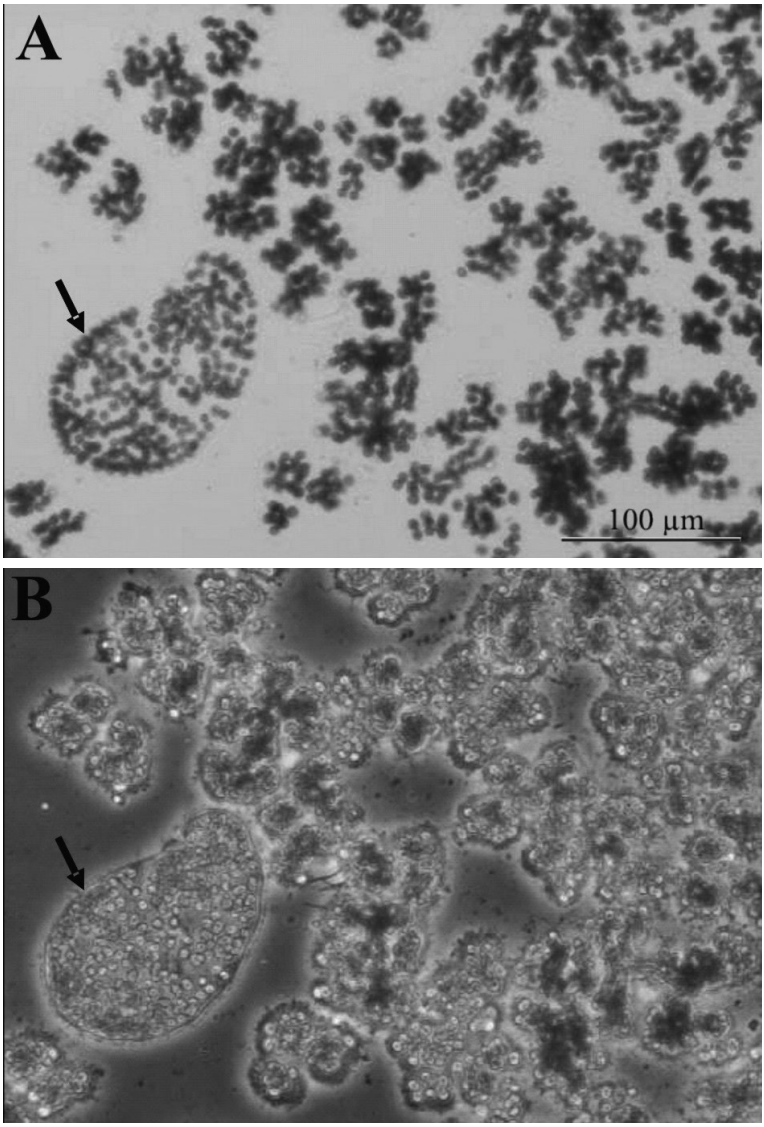

Fig. 2: Colonies of *Microcystis viridis* (right side of figure) and *Microcystis wesenbergii* (indicated by arrows) under (A) light and (B) phase contrast microscope, 200 x magnified, from Locality Boreci reservoir, 9.8.2006, photo: Tina Eleršek.

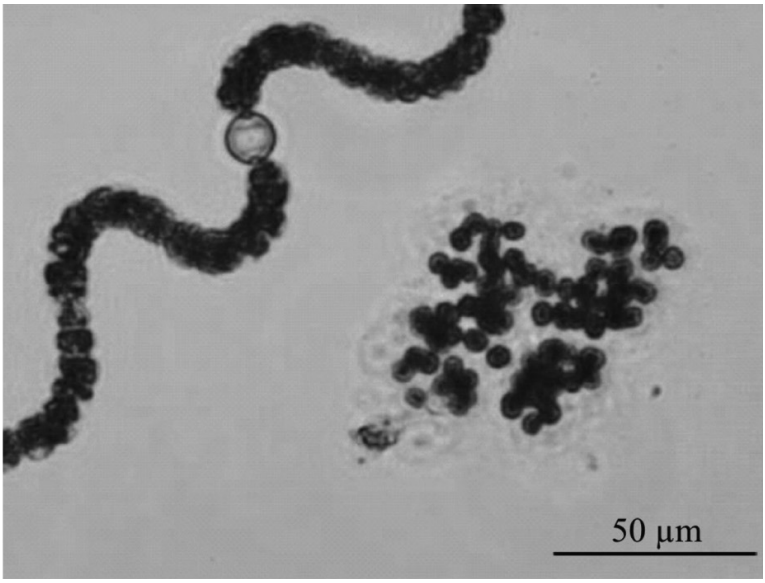

Fig. 3: Colonies of *Microcystis viridis* (right) and *Anabaena spiralis* (left) under phase contrast microscope, 400 x magnified, from Locality Boreci reservoir, 9.8.2006, photo: Tina Eleršek.

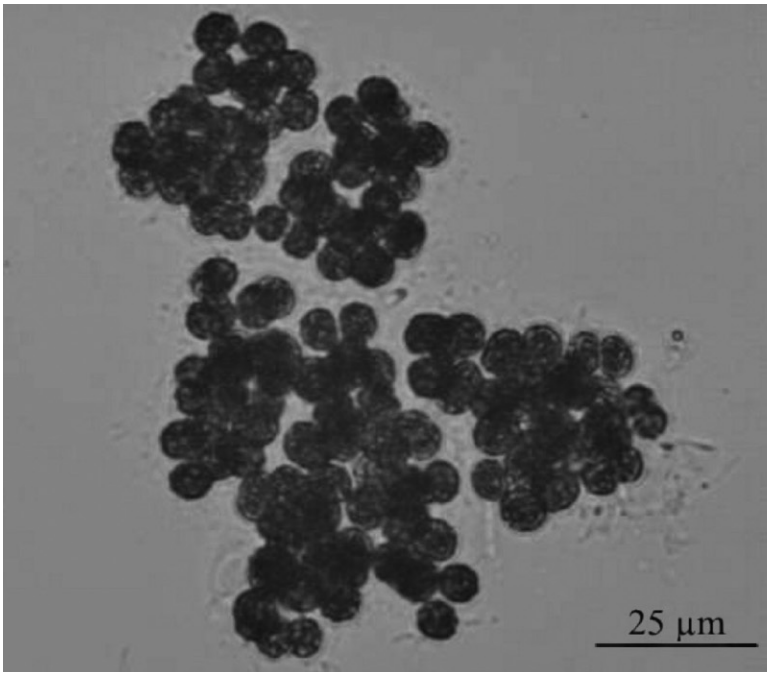

Fig. 4: Colonies of *Microcystis viridis* under phase contrast microscope, 600 x magnified, from Locality Boreci reservoir, 9.8.2006, photo: Tina Eleršek.

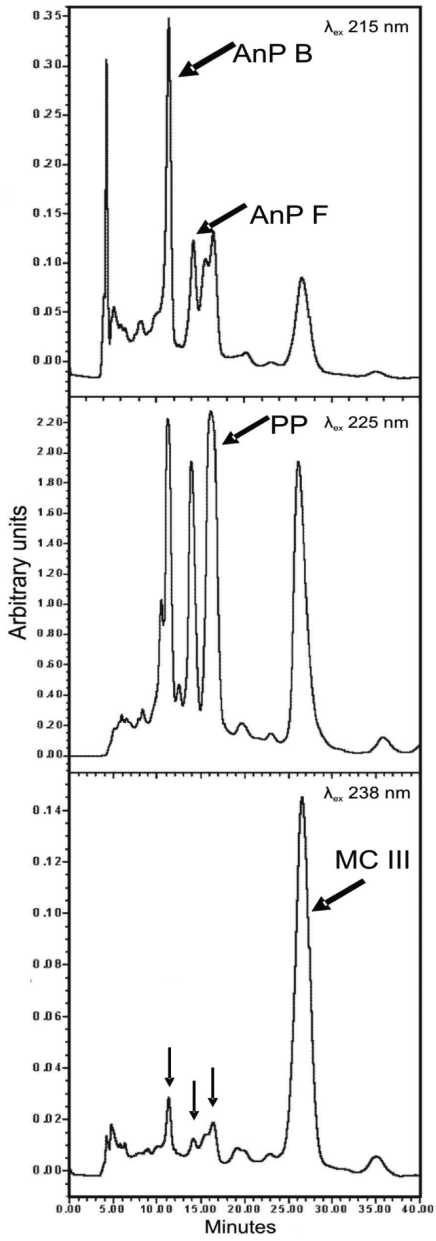

Fig. 5: HPLC chromatogram of *Microcystis viridis bloom* extract run from a preparative column using isocratic elution with methanol: phosphate buffer 50:50 (v/v). The diagrams show the elution pattern monitored at three different wavelengths: 215, 225 and 238 nm. MC is clearly visible at the characteristic λ_{max} of 238 nm, while the other three cyclic peptides are seen only as minor peaks (vertical arrows in the lowest panel). PP BL, AnP B and AnP F are better detected at lower wavelengths (upper two panels). AnP B = anabaenopeptin B; AnP F = anabaenopeptin F; PP = planktopeptin BL 1125, MC = unidentified microcystin

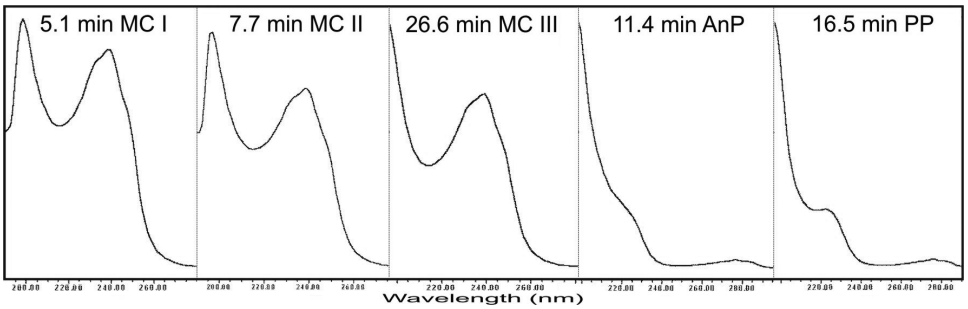

Fig. 6: The typical absorption spectra of five cyclic peptides from *Microcystis viridis bloom* at their characteristic retention time, marked in the upper part. AnP = anabaenopeptin; PP = planktopeptin BL 1125, MC = unidentified microcystin.

of *M. viridis* in the bloom in the first half of the August in reservoir Boreci (Fig. 1). After 8 days, *M. viridis* was found in two neighbouring reservoirs also, Hotinja vas and Podgrad. Changes in bloom sample structure were very fast; in just 8 days we observed different bloom composition (Table 2, Figs. 2 and 3). Cells were found in colonies (Fig. 4) with limited, more or less refractive mucilage, best seen under phase contrast microscopy (e.g. Fig. 2). The average diameter of cells was 4–7 µm. The contents of chlorophyll *a* of cyanobacterial bloom from Boreci reservoir were similar, 320 µg/l (9.8.2006) and 340 µg/l (17.8.2006). HPLC analysis showed that *M. viridis* bloom (from 9.8.2006) contains three MC and three non-toxic cyclic peptides, two anabaenopeptins and plaktopeptin BL1125 (Fig. 5), which have important roles in bloom lysis (Sedmak & Eleršek, 2005, 2006). All the cyclic peptides have characteristic absorption spectra (Fig. 6). Their content varied in the range of 2.3–6.6 mg g^{-1} of cellular dry weight. As found in previous studies (Sedmak & Kosi, 1997; Sedmak & Eleršek, 2005, 2006; Sedmak & al., 2007), the content of cyclic peptides was high enough to cause bloom lysis. Interestingly, this fact was confirmed by field observation; bloom composition not only changed, but, after 8 days, there was no visible cyanobacterial bloom on the reservoir surface, although no heavy rain or wind was detected during this period. The discovery of *M. viridis* bloom in Slovenia is very important, since toxic bloom constitutes a threat all over the World.

Conclusion

The presence of the cyanobacterial bloom of *Microcystis viridis* (A. Braun) Lemmermann is reported for the first time in Slovenia. Cells were found in colonies with refractive mucilage. The content of cyclic peptides (three microcystins, two anabaenopeptins and planktopeptin BL 1125) in the cyanobacterial bloom was high enough to cause bloom lysis. This fact was also confirmed by field observation. The discovery of *M. viridis* bloom in Slovenia is very important, since toxic bloom constitutes a threat all over the World.

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A biology teacher – a second career choice

Učitelj biologije – alternativna izbira poklica

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Abstract. For several years now, Slovenia has been facing a shortfall of interest in science studies. Some argue that the principal reason for this lies with inadequately trained science teachers in primary and secondary schools. We set out to find the reasons why double degree students of biology (Chemistry-Biology or Biology-Home Economics) at the Faculty of Education chose to become biology teachers, and outline certain guidelines for pedagogical work with students. The results of our survey show that the number of secondary school graduates whose first choice is either of the two biology teacher study programmes at the Faculty of Education is declining. Students who selected the programme as their second choice are mainly those who did not have enough credit points for their first options, i.e. medicine, dentistry, biochemistry, veterinary sciences or biology at the Biotechnical Faculty. Most of the students who decide to become biology teachers are female. The main motives are the students' fondness for biology and the desire to work with children. Students (regardless of which study programme they select) have fairly similar views on teaching biology. They believe that teachers should introduce practical work in the classroom, have good relationships with students, and are experts in their field (biology). Students in general did not provide innovative ideas about teaching methods which would not be boring or uninteresting. Their ideas reflect their own experience of teachers, which is why it is important that they are included in work with pupils as early as possible. In this way they can gain their own direct experience of teaching, which helps them to build their own unique idea of what a biology teacher should be. Also highly qualified inservice teachers (mentors) should take part in education of future biology teachers. Inservice teachers could according to functioning of future teachers' in the classroom propose to university teacher trainers what knowledge do those future teachers lack and should gain in a faculty setting. On the basis of this information introductory science courses could be better accommodated to the needs of future teachers.

Keywords. student science teacher, teacher's qualities, good biology teacher

Introduction

For several years now, Slovenia has been facing a shortfall of interest in science studies. This is also shown even through the secondary school students' choices of science subjects at the school-leaving examination (Zupanc & al. 2006). Likewise, interest in studying to be a science (biology) teacher has fallen. For example, data obtained from the Registration Office of the University of Ljubljana (Vpis 2008) show that the share of science teacher candidates opting

for Chemistry-Biology or Biology-Home Economics programmes at the Faculty of Education in Ljubljana as their first career choice has been decreasing year by year (Vpis 2008). Most telling is the information about students of Chemistry-Biology, as less than half of the students in the academic years 2004/05, 2005/06 and 2006/07 selected this programme as their first choice. These students are potential candidates for teaching biology subjects at primary-school level (grades six to nine) and in vocational secondary schools.

Teachers in primary schools influence (co-shape) pupils' attitude toward science subjects (Lederman 2008). It is important that a student who chooses to work as a teacher makes this decision intentionally and wilfully (Draper 2001) and should also be aware that the position of teachers in society is changing (Ben-Peretz 2001, Robinson & Mcmillan 2006). Therefore, the motive for science teacher candidates selecting this course as their second career choice should be investigated. Cencič (2000) finds that the main motives for students choosing to work as teachers are fondness for teaching, the desire to work with children and various altruistic motives. But some would disagree that these reasons are satisfactory (Vilhar 2005). Some might believe that teachers' intelligence is strongly related to their success in teaching. However, a certain minimal level of intelligence is necessary for effective teaching, but beyond this point, the intelligence of teachers may not be significantly related to pupils' learning outcomes (Ausubel & al. 1978, Smith 2008). The important characteristic of a teacher's effectiveness is their ability to adapt the communication of ideas to the pupil's level of intellectual maturity and degree of sophistication of the subject matter (Ausubel & al. 1978). In the article of Demirbolat (2006) some innovative teacher roles are listed.

Often public opinion is that teachers are one of the key determinants of pupils' interest in certain subject. For example Vilhar (2005) believes that the declining interest in science studies (biology) in Slovenia could be attributed to the present science teachers. This argument could be partly supported by findings of Japelj & al. (2005). They found that Slovenian high school students are more interested in studying social sciences than science (e.g. biology and chemistry). Apparently, the main reasons are that science lessons lack experiments and contents that would be connected to everyday life. Primary school students show negative attitude toward science, too. In order to improve enthusiasm for science and technology among the latter some primary school teachers' joined the Pollen project (Pollen 2008). The main aim of this project is to stimulate and support inquiry based science education in European primary schools.

Not only Slovenia is facing a shortfall of interest in science studies. For example Crawford (2000, 2007) reports that science teachers in the USA have become acquainted with alternative approaches (National Research Council 2001) to science teaching so that they could overcome the problem of students' disinterest in science subjects. Weld & Funk (2005) believe that teachers have to take the step from believing that life sciences should be presented as a compendium of facts driven by a textbook to viewing them as essentially related to learners' lives through active investigation. We believe that future science teachers should also make these steps to overcome traditional-oriented conceptions about teaching science and learning. In Slovenia, some changes have been made to both the process of educating science teachers and science curricula in order to overcome disinterest in science subjects among students. So far, no reform has yielded the expected result – increased interest in science. Other countries face similar problems, stressing that teacher's ideas about subject matter, teaching and learning do not change easily or rapidly, while teachers do not tend to risk changing their own practice, which is rooted in practical knowledge built up over the course of their careers (see Van Driel & al. 2001). Therefore, to avoid these problems, science teacher candidates should gain experience in teaching, collaborating with other teachers, parents, the school system, etc. as early as possible, so that they can learn about the challenges of this occupation. When in practice they should work with highly experienced inservice teacher (mentor) in order to get or to improve teaching skills. Parker-Katz & Bay (2008) believe that mentors' experiences, observations, and interpretations are very important in understanding the relationship between teaching and learning and also helping a future teacher to see

this relationship as well. Early work with pupils should give a future teacher adequate experiences to overcome his /her school science experiences. Lederman (2008) states, that a properly trained teacher creates the magic, colours the learning, and resonates with the students.

In order to design guidelines for the education of student science teachers in the field of biology in Slovenia so that they would be able to negotiate the challenges of their profession successfully, we studied the students' motives for their decision to study science subjects (Chemistry–Biology and Biology–Home Economics at the Faculty of Education in Ljubljana). In addition to their decision to become science teachers, we focused on how students see themselves in their future role. On the basis of the data we collected, we propose some guidelines for educating prospective biology teachers in Slovenia.

Method

Participants

In our study we focused primarily on first-year students majoring in Chemistry-Biology and Biology-Home economics at the Faculty of Education in Ljubljana who, after graduating, can teach biology subjects at primary-school level (grades six to nine) and in vocational secondary schools.

The research included students studying in 2000/01, 2001/02, 2002/03, 2004/05 and 2006/07 – a total of 247 student teachers. We did not include students in the academic years 2003/04 in 2005/06 in the research.

Procedure

Before the beginning of lectures on the Methodology of biology education, the first-year students of Chemistry-Biology and Biology-Home economics were asked to complete a questionnaire about (1) the reasons that had led them to take these courses, (2) the qualities of a good biology teacher, and (3) how they see themselves in the role of a biology teacher, which ensured that their answers were not influenced by the lectures. The questions were open-ended, in order not to restrict or guide responses in any way. The reason for this was to obtain a realistic image of students' decisions to study biology. Participants were told that there was no right or wrong answer, but that the focus was on their beliefs, and that their answers should reflect their beliefs as succinctly as possible, and that the length of their responses was up to them (Haritos 2004). Students were also asked to indicate their age and gender. The questionnaire was anonymous.

We sorted students' answers, following Ivanuš Grmek & Javornik Krečič (2005), into the following categories: **altruistic reasons** *(I wish to work with children; I wanted to become a teacher; I like children.*); **material reasons** (*attractive working conditions: holidays, opportunities for further education, seminars, salary*); **alternative reasons** (*It's a coincidence that I'm studying this; I couldn't take the course I wanted to*); **reasons of self-fulfilment** (*As a teacher I will be able to use all my abilities; I will be a model for children and young people.*); and **personal aspirations and stereotypes** (*It means a lot to me to have this level of education; Teaching is a woman's profession; I come from a family of teachers.*). We added two additional categories: **fondness for biology** (I like biology.) **and teacher's influence** (My biology teacher inspired me.).

We arranged the answers to the questions "What makes a good biology teacher?" and "How do you see yourself in the role of a teacher?" in the following categories: **relationship to nature and organisms** (*a person who loves nature, who likes plants and animals, who 'breathes' with nature, etc.*); **professional commitment** (*wholeheartedly committed to the profession, enjoys work, lives for his/her profession, etc.*); **personal characteristics** (*kind, interesting, fun, happy, has a sense of humour, strict, but not too strict, consistent, ambitious, etc.*); **expertise** (*knows a lot, proficient in his/her subject, discusses current topics, very experienced, etc.*); **teaching proficiency** (*teaches a great deal, gives students a lot of general knowledge, arouses students' interest in living organisms, his/her students are successful in competitions and national examinations, etc.*); **relationship to children** (*has a great feeling for working with children, listens to pupils, pupils can trust him/her, they are not afraid of him/her, always prepared to assist or help his/her pupils, considers his/her pupils' needs, etc.*); **teaching**

approaches (*explains well, includes practical work in the classroom, works with concrete materials, etc.*)

We analysed all data with SigmaStat, Version 3.1. Since the research included more females than males, we did not make comparisons according to gender.

Results and Discussion

Gender of science teacher candidates

Since the mid-1990s, concern has been expressed about the feminisation of the labour force in teaching. Some experts argue that in recent years the number of male students interested in becoming teachers has decreased (White & al. 2006). Our data support these findings, in all academic years included in the research, only 8% of the 247 science teacher candidates were male, compared to the 92% share of female students (Tab. 1). This may signal that this occupation has become less attractive to men because of greater potential earnings or status in other comparable professions (Wylie 2000). However, Rots & al. (2002), find that society does not seem to perceive the growing number of female teachers as a problem for the quality of education. Their findings are significant, as it is known that a teacher's effectiveness is usually estimated through the learning outcomes of students (Ausubel & al. 1978, Smith 2008). The findings of Rots & al. (2002) are also backed by Driessen (2007), who confirmed that a teacher's gender has no effect on the achievement, attitudes or behaviour of pupils (boys and girls).

Selecting teaching as a career

In order to draw up teaching methodology guidelines for the biology study programme, we studied the choices made by secondary school students (who have passed the school-leaving exam) to take up biology teaching. A comparison between the number of students enrolling in the two study programmes and their wishes shows discrepancies between the data obtained from VPIS (VPIS 2008) and the data obtained in our research (Tab. 1).

Both show that the share of students whose first choice were Chemistry-Biology or Biology-Home Economics courses is falling, the decline in the academic years included in our research is not as substantial as indicated by the VPIS data. According to all the data, the percentage of students who originally opted for these two study programmes was lowest in the 2006/07 academic year. Our research also recorded fewer students per academic year than VPIS. The reason for this was most possibly the fact that not all students recorded by VPIS actually attended lectures,

Table 1: Selecting the double degree biology study programme at the Faculty of Education in Ljubljana by years

Tabela 1: Izbira študija biologije z vezavama na Pedagoški fakulteti v Ljubljani po letih

Academic year	N_t	(N_m)	Selecting the double degree biology study programme					
			first choice		second choice		indeterminate	
			(%)	N	*(%)*	N	*(%)*	N
2000/01	53	(7)	*17,0*	9	*28,3*	15	*54,7*	29
2001/02	52	(4)	*19,2*	10	*28,8*	15	*51,9*	27
2002/03	44	(4)	*25,0*	11	*29,5*	13	*45,5*	20
2004/05	45	(2)	*8,9*	4	*28,9*	13	*62,2*	28
2006/07	53	(2)	*24,5*	13	*35,8*	19	*39,6*	21
Total	247	(19)		47		75		125

N_t – total number of students, N_m – number of male students.

N_t – število vseh študentov, N_m – število študentov moškeg spola.

seminars and practical work, for which reason we could not have included them in our survey. It is also possible that some students dropped out, while some resumed their studies in a following year.

Reasons for students selecting the double degree biology study programme at the Ljubljana Faculty of Education

Students studying between 2000 and 2007 expressed similar reasons for studying biology together with either of the two other subjects (Tab. 2). The most common reason was that they were interested in biology. The second and third most common reasons were either altruistic, or as an alternative to their first choice. Twenty or more per cent of students of each study year stated alternative reasons for taking up the study of biology. This, however, does not correspond to the data on the selection of study programmes included in Tab. 1. We presume that some indeterminate students stated their real reasons (including alternatives) for choosing to teach. We then asked students who stated that teaching biology was their alternative choice about their first choice. 56 of the 75 students stated their first choice. We found that 43 of them had listed a natural science subject as their first choice, while 13 had originally wanted to study social sciences. The natural sciences included medicine, dentistry, biochemistry, microbiology, veterinary sciences and biology at the Biotechnical Faculty, while social sciences included geography, psychology, art education, elementary education, social psychology or sociology. Some students mentioned that although studying to become a teacher was an alternative, they had always found biology very interesting. Some stated that they wanted to become teachers (*author's note:* geography, art, elementary education).

Table 2: Students' reasons for selecting the teaching profession
Tabela 2: Razlogi študentov za izbor učiteljskega poklica

Categories	Academic years									
	2000/01		2001/02		2002/03		2004/05		2006/07	
	(%)	N_s=70	*(%)*	N_s= 76	*(%)*	N_s= 66	*(%)*	N_s= 67	*(%)*	N_s= 93
Fondness for biology	*40,0*	28	*40,8*	31	*36,4*	24	40,3	27	*37,6*	35
Alternative reasons	*21,4*	15	*23,7*	18	*19,7*	13	*22,4*	15	*22,6*	21
Altruistic reasons	*17,1*	12	*27,6*	21	*31,8*	21	*26,9*	18	*34,4*	32
Reasons of self-fulfilment	*15,7*	11	*5,3*	4	*4,5*	3	*6,0*	4	*4,3*	4
Teacher's influence	*5,7*	4	*1,3*	1	*6,1*	4	*3,0*	2		0
Personal aspirations and stereotypes		0		0		0		0	*1,1*	1
Material reasons		0	*1,3*	1	*1,5*	1	*1,5*	1		0

N_s = number of statements. Students were allowed to mention more than only 1 reason for selecting the teaching profession at the Faculty of Education in Ljubljana.
N_s = število navedb. Študenti so lahko navedli več kot 1 razlog za izbor študija na Pedagoški fakulteti v Ljubljani.

The number of answers classified as altruistic reasons increased two-fold between the study years 2000/01 and 2006/07, with the most common being "I wish to work with children". The case with the 'self-fulfilment' group was quite the opposite, however, as their number fell by over three times between 2000/01 and 2006/07. Personal aspirations or material reasons, however, did not play a decisive role in students' decision to study biology at the Ljubljana Faculty of Education, as they were the least commonly stated motives in each study year. None of the students mentioned the 'benefits' of working as a teacher,

Table 3: Students' beliefs of what makes a good biology teacher
Tabela 3: Mnenja študentov o tem, kakšen je dober učitelj biologije

Categories	Selecting the double degree biology study programme						Chi-Square Test	
	first choice		second choice		indetermi-nate			
	(%)	N_s	(%)	N_s	(%)	N_s	sig. χ^2 (2)	*p*
Relationship to children	*2,0*	5	*4,0*	10	*8,5*	21	1,175	0,556
Relationship to nature and organisms	*0,4*	1	1,6	4	*4,5*	11	2,743	0,254
Personal characteristics	*3,6*	9	*5,3*	13	*11,3*	28	0,788	0,674
Teaching approaches	*17,4*	43	*21,9*	54	*42,9*	106	8,677	0,013
Teaching proficiency	*2,4*	6	*3,6*	9	*6,1*	15	0,021	0,990
Expertise	*4,0*	10	3,2	8	*6,1*	15	3,214	0,200
Professional commitment	*1,6*	4	*2,4*	6	*3,6*	9	0,097	0,953

N_s – number of statements.
N_s – število navedb.

such as working hours, public exposure, more family time, holidays, further training, etc. as having influenced their choice. In fact, Richardson and Watt (2005) found something completely different in their research: prior considerations, career fit, time for family and financial reward were the most important factors for selecting teaching as a career. The reason for such opposite views may lie in the social differences between students of different countries and also in their expectations regarding employment.

The results of this study show that although some teacher candidates did not select teaching as their first choice, some of them have a positive attitude towards biology or teaching.

What are the characteristics of a good biology teacher?

One looks back with appreciation to the brilliant teachers, but with gratitude to those who touched our human feelings. The curriculum is so much necessary raw material, but warmth is the vital element for the growing plant and for the soul of the child. (Carl Jung)

Table 4: How a good biology teacher teaches?
Tabela 4: Kako poučuje dober učitelj biologije?

Categories	Selecting the double degree biology study programme					
	first choice		second choice		indetermi-nate	
	(%)	N_s	*(%)*	N_s	*(%)*	N_s
Practical work (experiments, hands-on approach to learning)	*17,0*	8		0		0
Interesting lessons	*4,3*	2	4,0	3	4,0	5
Draws students' attention	*6,4*	3	*9,3*	7	7,2	9
Students like to take an active part in lessons.	*4,3*	2	*2,7*	2	4,8	6
Usage of concrete materials (plants, animals …)	*10,6*	5	21,3	16	20,0	25
Explanations (interesting, good, simple...)	*61,7*	29	*40,0*	30	49,6	62
Field work	*4,3*	2	2,7	2	2,4	3

N_s – number of statements.
N_s – število navedb.

In the study, we attempted to sum up the qualities of a good biology teacher on the basis of students' opinions. We sorted students' answers into categories (Tab. 3)

Regardless of their choice of study programme, students provided answers that fit any of the categories, except for the 'teaching approaches' category. The most frequent response in the latter was that a good biology teacher provides good, interesting and simple explanations (Tab. 4).

The most frequent answers in this category, from students for whom biology was the first choice, were those that emphasised the importance of good explanations, and they often stressed the importance of practical work. We find it interesting that none of the students who stated that they had selected biology as their second choice or were indeterminate about career choice, mentioned the importance of practical work (e.g. conducting live experiments, offering a hands-on approach to learning) for the teaching process. They did, however, state that a good teacher is one who uses concrete materials in the classroom. Other statements are shown in Tab. 4. In addition to a variety of teaching approaches, students also pointed out several character traits of a biology teacher. In their view, a good teacher should be kind, interesting, relaxed, fair, fun, authoritative and a good disciplinarian. They also stressed two other qualities of a good teacher: good relations with students and expertise (Tab. 3). Although we assume that our data reflect the respondents' actual beliefs, it is possible that they gave "socially correct answers" (Haritos 2004). The similarities between the answers of the 'first choice' and 'second choice' groups could be attributable to the fact that they had not had any direct experience of teaching, but had only experienced 'good' teachers as pupils themselves. We believe that when students described the qualities of a good teacher, they probably drew on their experience of biology teachers in primary and/or secondary schools, pointing out what they most liked about them or felt they lacked.

To summarise: a good biology teacher does not present dull information; includes practical work in the classroom; has good relationships with students, and possesses a high degree of expertise.

How do you see yourself in the role of a teacher?

Regardless of their choice of study programme, students saw themselves as teachers in similar ways, and there were no statistically significant differences between the groups. The majority of answers from both groups fell into the 'teaching approaches' and 'personal characteristics' categories (Tab. 5). Their answers were similar to responses to the question "What makes a good biology teacher?". They claimed that they would provide interesting, easy to understand and simple explanations. Given the fact that in their analysis Japelj & al. (2005) find that secondary school students believe science lessons do not have enough experiments and that lessons

Table 5: How teacher students see themselves in the role of a teacher?
Tabela 5: Kako študenti vidijo sebe v vlogi učitelja?

Categories	Selecting the double degree biology study programme						Chi-Square Test	
	first choice		second choice		indetermi-nate			
	(%)	N_s	(%)	N_s	(%)	N_s	sig. χ^2 (2)	p
Relationship to children	*1,4*	3	*5,3*	7	*10,0*	8	0,667	0,716
Relationship to nature and organisms		0	*0,8*	1		0	*2,303*	0,316
Personal characteristics	*6,1*	13	*14,3*	19	*30,0*	24	*1,829*	0,401
Teaching approaches	*8,5*	18	*18,0*	24	*60,0*	48	*0,916*	0,633
Teaching proficiency	*2,8*	6	*3,8*	5	*8,8*	7	*2,657*	0,265
Expertise	*0,5*	1		0	*3,8*	3	*1,789*	0,409
Professional commitment	*0,9*	2	*0,8*	1	*2,5*	2	*1,747*	0,479

should be more connected with everyday life, we expected that most of the respondents would state that they would foster learning through experimental work; however, only 12.6% of them mentioned this. The most common answer from the majority was that they wish to provide good explanations. Very few mentioned the importance of the teacher's expertise, commitment to the job, qualification to work with children, etc. (Tab. 5). We therefore believe it is important that during their university years, teacher candidates should acquire experience in teaching methods which are not based only on instruction, since it is well known that as teachers they will tend to teach in the way they were taught when they were students (Dana & al. 1997, Tsai 2002). Student science teachers acquire a wide range of experience in working with children; therefore we believe that they should start training in a school environment as soon as possible. During training, trainers should consider the students' 'idealised' image of teaching, and help them create a real one. Therefore it is important that a trainer (mentor) who works with a student science teacher is highly experienced (Parker-Katz & Bay, 2008).

Two respondents (biology as the second choice and undecided) did state, however, that they do not intend to work as teachers after completing their studies and, surprisingly, 26 respondents (6 first choice, 10 second choice, 10 indeterminate) stressed that they could not yet see themselves as teachers. Very few students gave answers related to marking, preparation, administration, working with parents, upbringing, or working with difficult children and children with special needs, which is understandable, since students have not had any teaching experience.

According to Morais & al. (2005), we should not overlook the fact that teachers' development depends not only on the characteristics of the teacher training processes, but is influenced by many personal, social and occupational factors. Personal characteristics, the working environment at school, relations between colleagues, relations with parents and the community influence teachers' development.

Conclusion

In Slovenia, there has been much debate about the declining interest in science studies. The lack of interest is shown clearly in secondary school students' choices of optional subjects at the school-leaving examination (at the end of secondary school), where the share of science subjects has been falling by about one per cent per year (Zupanc & al. 2006). Despite changes in the science and biology curricula in primary and secondary schools and efforts (seminars, training courses, etc.) to make teachers adopt alternative teaching methods, science subjects are losing popularity.

It is alarming that the share of students whose first choice university course was either Chemistry-Biology or Biology-Home economics is falling (Tab. 1). Among the main reasons for the lack of interest in science studies is not only that young people prefer jobs which enjoy a higher social status, and promise a better starting salary and mass media interest, but also the fact that the teaching profession has largely lost the reputation it once had. The position of teachers in society is changing (Ben-Peretz 2001). We found that the number of male students interested in becoming teachers has decreased, probably because of greater potential earnings or status in other, comparable occupations (Wylie 2000). However, increasing number of female science teacher candidates shouldn't pose a problem, since it is known that a teacher's gender has no effect on the pupils' achievement (Rots & al. 2002, Driessen 2007).

We found encouraging that some secondary school students who opt for science studies after having failed to qualify for their first choice subject answered that they like either teaching or biology. Despite their ideas about teaching being a more or less one-way communication process, they too could one day become good science or biology teachers, provided they acquire the necessary knowledge in a good, well-structured course. It should be considered that these students should have additional lessons with university teacher trainers in order to evaluate progress gained during the study and practice.

We asked prospective teachers what qualities a good biology teacher should have. Their

answers surprised us, as they replied that a good teacher was one who can explain subject matter in an interesting way. Despite having experienced the school system at primary and secondary levels, students failed to provide innovative ideas about teaching which are not merely dull and uninteresting. However students' answers could be supported by Dana & al. (1997) and Tsai (2002) who stressed that such view could stem from students' own school science experience.

In the light of students' answers, one could conclude that university teacher training courses adapt much too slowly and inadequately to the needs of pupils and students.

Demirbolat (2006) suggests that individualistic approach in teacher training should be emphasized because the development of future teacher values are more influenced by factors like personal attributes and environment in which person grew-up than the programmes that train teachers. Individualistic approach is intensely implemented in biology part of programmes of Chemistry-Biology and Biology-Home Economics. However, in the past years more intention was emphasised on the form of students' participation in the practice and less on selection of teacher trainers (mentors), who can influence teaching skills of a future teacher (Parker-Katz & Bay, 2008). Therefore, it should be considered to reestablish a network of highly qualified teacher mentors who would present the linkage between the faculty (study programme) and schools (teaching and learning in practice). Highly qualified inservice teachers could according to function of future teachers' in practice propose what knowledge do those future teachers lack and should gain in a faculty setting. Teachers who draw up teacher training could on the basis of this information re-examine and redesign introductory science courses to better accommodate the needs of future teachers (National Research Council 2001, p. 118).

Furthermore, teacher trainers preparing new teachers for schools should be aware that schools today are very different from the schools they themselves experienced as young people (Robinson & Mcmillan 2006). Curricular reforms do not present a bright future for prospective biology teachers unless they focus on the pedagogical understanding of teaching based on solid scientific foundations. On the one hand, expertise alone does not guarantee good teaching, and on the other, good teaching skills coupled with a lack of expertise cannot develop knowledge based on understanding. Perhaps well-trained teachers are the key to dispelling the myth that science (biology) is merely a set of dull facts, but is based on understanding phenomena and life that surround us.

In addition to this, probably some system changes would be welcome in order to make the profession truly desirable for teacher candidates (Demirbolat 2006) and to attract good students to make a decision to become teachers and even more to become a good and perspective science (biology) teachers.

Povzetek

V Sloveniji se že nekaj let zapored soočamo z upadom zanimanja za študij naravoslovnih ved. Po mnenju nekaterih k takšnemu stanju prispevajo neprimerno usposobljeni učitelji naravoslovnih predmetov v osnovnih in srednjih šolah. Pri študentih dvopredmetnega študija biologije z vezavama na Pedagoški fakulteti v Ljubljani smo v študijskih letih (2000/01, 2001/02, 2002/03, 2004/05, 2006/07) preverili razloge za odločitev za poklic učitelja biologije in izpostavili nekatera izhodišča za pedagoško delo s študenti pri predmetu Metodika biološkega izobraževanja. V raziskavo smo vključili študente 1. letnika (n = 247). Študenti so izpolnili vprašalnik o delu učitelja biologije in naravoslovja pred začetkom predavanj, ki so povezana z metodiko biološkega izobraževanja. S tem smo zagotovili, da so študenti na vprašanja odgovarjali neodvisno od vsebin, ki jih slišijo na predavanjih ali vajah.

Rezultati raziskave so pokazali, da upada število tistih dijakov, ki po končani maturi pod prvo željo izberejo program učitelja biologije z vezavama na Pedagoški fakultete. Med tistimi, ki se odločijo za ta študij pod drugo željo pa je večina takih, ki se ni uspela vpisati na medicino, stomatologijo, biokemijo, veterino ali na študij biologije na Biotehniški fakulteti. Za poklic učitelja biologije se večinoma odločajo dekleta. Prevladujoča motiva za izbor študija sta navdušenje študentov do biologije in želja po

delu z otroki. Študenti (ne glede na izbor študija) imajo zelo podobne predstave o poklicu učitelja biologije, ki naj bi po njihovem mnenju v pouk uvajal praktično delo, se razumel z učenci in bil strokovnjak na svojem (biološkem) področju. Študenti praviloma niso navajali inovativnih idej o poučevanju, ki ni zgolj dolgočasno in nezanimivo. Njihove predstave so odraz lastnih izkušenj z učitelji, zato je pomembno, da se študenti dovolj zgodaj vključijo v delo z učenci. Tako lahko pridobijo lastne in neposredne izkušnje o poučevanju, ki jim pomagajo graditi samosvojo (realno) podobo učitelja biologije (naravoslovja). Namreč, učitelju obvladovanje stroke še ne omogoča kvalitetnega dela v razredu. Prav tako pa dobra učiteljeva pedagoška podlaga brez strokovnih kompetenc ne more pri učencih razvijati znanja z razumevanjem.

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Biology Teachers' Conceptions about Nature and Environment - Two Fundamental Concepts of Education for Sustainable Development

Predstave učiteljev biologije o naravi in okolju - dveh temeljnih pojmih vzgoje in izobraževanja za trajnostni razvoj

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Abstract. Education for Sustainable development (ESD) is an important strategy in achieving environmental improvement. Educators, such us biology teachers, have an important role to play. This article explores biology teachers' conceptions and ideas about nature and environment, two basic conceptions in ESD. The study involved 105 biology teachers working in primary schools in Slovenia. The participating biology teachers completed a word association questionnaire. The analyses of the data show that the dominant conception of biology teachers concerning nature is biophysical. Nature is seen as non-human environment used by humans only as place of sport, recreation, beauty, quietness and rest. Dominant dimensions of environment are biophysical dimension, dimension of destruction and technostructure dimension. Environment is seen as place more dominated by human activities, infrastructure and consequences of pollution and degradation. Some similarities in teachers' conception of nature and environment indicate conceptual confusion. Some teachers obviously think that the concept of environment is identical with that of nature.

Key words. environment, nature, biology teacher, concepts, Education for Sustainable Development

Izvleček. Vzgoja in izobraževanje za trajnostni razvoj (VITR) je pomembna strategija za dosego izboljšanja stanja v okolju. Učitelji biologije imajo v tem procesu pomembno vlogo. Za ugotavljanje učiteljevih predstav o naravi in okolju, dveh temeljnih konceptov VITR, smo uporabili metodo prostih asociacij. V raziskavi je sodelovalo 105 učiteljev biologije, ki učijo v slovenskih osnovnih šolah. Ugotavljamo, da prevladujoča biofizična predstava o naravi. Narava je v predstavah učiteljev biologije prostor, kjer človek ni prisoten, kamor se le občasno zateče po mir, lepoto, počitek ali v športno-rekreativne namene. Prevladujoče dimenzije predstav o okolju so tri: biofizična, tehnično-infrastrukturna ter dimenzija degradacije. Okolje zaznavajo kot prostor, ki ga s svojimi dejavnostmi, infrastrukturo in degradacijo zaznamuje človek. Nekatere podobnosti v percepciji narave in okolja kažejo na zmedo v razlikovanju teh dveh pojmov. Nekateri učitelji očitno enačijo pojma okolje in narava.

Ključne besede. okolje, narava, učitelj biologije, predstave, vzgoja in izobraževanje za trajnostni razvoj

Uvod

Raziskave, ki beležijo, kategorizirajo in interpretirajo predstave in ideje pojmov v biologiji so zelo pogoste (npr. Ali, 2002; Braund, 1991; Prokop et al. 2007; Shepardson, 1997; Tekkaya, 2003; Torkar in Bajd, 2006). V tovrstnih raziskavah se poleg kvalitativnih metod raziskovanja uporabljajo tudi odprta vprašanja, pred-testi in po-testi (Summers et al. 2000), pojmovne mape (Munson, 1994) ter metoda prostih asociacij na ključne besede (Sato in James, 1999).

Združeni narodi so obdobje med letoma 2005 in 2014 razglasili za desetletje vzgoje in izobraževanja za trajnostni razvoj (*ang.* Dacade of Education for Sustainable Development) in s tem izpostavili, kako pomembno je danes to vprašanje v svetu. Tudi Bela knjiga o vzgoji in izobraževanju v Republiki Sloveniji (1995) postavlja med temeljne vzgojno-izobraževalne cilje vzgojo za zdrav način življenja in razvoj odgovornega odnosa do okolja in narave. To seveda pred učitelje postavlja pomembno nalogo. Vzgoja in izobraževanje za trajnostni razvoj (VITR) je namreč interdisciplinarno področje, kjer se že osvojene besede iz biologije, kot tudi širše iz naravoslovja, humanistike in družboslovja, prikažejo v novem kontekstu ter s tem v novem pomenu. VITR je v sredini devetdesetih let prejšnjega stoletja nasledila okoljsko vzgojo, ker slednja preprosto ni več ponujala potrebne širine za kompleksno obravnavo okoljskih vprašanj (World Conference on Education for All,1992).

Vse večji pojmoven razpon dobivajo besede, ki jih uporabljamo tako v strokovnem kot vse bolj tudi v vsakdanjem pogovoru (npr. okolje, narava, ekologija). Opisana kompleksnost neprestane prehodnosti pojmov postavlja pred učitelje nove in nove izzive. Ključne pojme in njihove značilnosti si zato pogosto vsakdo razlaga nekoliko drugače (Palmer in Neal, 1994).

Na področju vzgoje in izobraževanja za trajnostni razvoj je raziskav, ki bi beležile, kategorizirale in interpretirale predstave in ideje o temeljnih pojmih, kot so okolje, narava, okoljevarstvo, globalno segrevanje itd. razmeroma malo (Agelidou et al., 2000; Flogaitis in Agelidou, 2003; Loughland et al., 2000; Krömker, 2004; Palmer, 1994; Sato in James, 1999; Khalid, 2003). Agelidou et al. (2000) ugotavljajo, da imajo učenci in učitelji veliko težav prav s kompleksnostjo obravnavanih okoljskih problemov. Mausner (1996), Flogaitis in Agelidou (2003) in Krömker (2004) ugotavljajo, da naravo odrasli ljudje najpogosteje povezujemo z nečloveškimi entitetami, kot so drevesa, živali, travniki in gozdovi ter prostori, kjer ni moč zaznavati prisotnosti človeka. Medtem ko so estetske kategorije (npr. lepota), relaksacijske kategorije (npr. počitek, mir) in oskrbovalne funkcije narave (npr. les) manj pogosto omenjane. Naravo povezujemo tudi z neživimi naravnimi elementi in pojavi, kot so oblaki, voda ali kamenje. Krnel (2005) je ugotavljal razlike in podobnosti med besedama »čisto« in »naravno«. Ugotovil je, da gre za besedi v tesni medsebojni zvezi ali pa se besedi celo zamenjujeta in uporabljata kot sinonima. Pojmovanje besede okolje se v zadnjih desetletjih zelo hitro razvija, predvsem na račun očitno vse bolj zaznavnih okoljevarstvenih problemov. MacDonald (2003) pojasnjuje, da je sprva šlo za znanstveni pojem, ki pa se je v svoji »evoluciji«, tudi s pomočjo politikov, novinarjev, filozofov in umetnikov, razvijal in ga lahko danes opišemo kot zmes ekoloških, ekonomskih, estetskih in etičnih vidikov. Flogaitis in Agelidou (2003) sta v raziskavi, ki je zajela grške vzgojitelje predšolskih otrok, ugotavljala razlike v njihovem razumevanju predstav o okolju in naravi. Ugotavljata, da je beseda narava veliko »bolj obtežena« z emocionalno komponento kot beseda okolje. Slednja pa se pogosteje povezuje z degradacijo in onesnaževanjem.

V raziskavi želimo ugotoviti, kakšne so razlike v razumevanju pojmov narava in okolje med slovenskimi učitelji biologije. S tem želimo dobiti vpogled v dimenzionalno strukturo predstav ter njihovo frekvenčno zastopanost. Pojasniti želimo tudi glavne razlike v pojmovanju okolja in narave. Raziskovanje teh dveh predstav je zanimivo, saj gre za dva splošna (»makro«) pojma, ki sta podvržena različnim individualnim interpretacijam (Palmer in Neal, 1994). Po mnenju Sata in Jamesa (1999) sta ta dva pojma osrednjega pomena za doseganje ciljev VITR. Z ozirom na veliko odgovornost, ki jo imajo učitelji biologije pri implementaciji VITR (glej Bela knjiga o vzgoji in izobraževanju v Republiki Sloveniji, 1995) bi morebitne napačne predstave o pojmih narava in okolje lahko negativno vplivale na sam razvoj VITR v teoretičnem in praktičnem smislu.

Metodologija

Vzorec

V raziskavo je bilo vključenih 105 učiteljev biologije z vezavo, ki poučujejo v slovenskih osnovnih šolah. Učitelji biologije poučujejo v 8. in 9. razredu devetletke, ob ustrezni dokvalifikaciji pa lahko poučujejo tudi naravoslovje v 6. in 7. razredu. V našem vzorcu sta bili 102 učiteljici in 3 učitelji biologije.

Metoda

Z metodo prostih asociacij na ključne besede (Sato in James, 1999) smo ugotavljali predstave učiteljev o naravi in okolju. Vsak učitelj je lahko zapisal do deset prostih asociacij na navedeni ključni besedi. Oblika navajanja asociacij ni bila posebej določena. Učitelji so jih navajali po alinejah, v celih stavkih ali v obliki miselnih vzorcev.

Zbiranje podatkov

Anketirani so vprašalnik izpolnjevali na stalnih strokovnih izobraževanjih, seminarjih in v šolah. Sodelovanje v raziskavi je bilo anonimno. Da bi dosegli čim večji odziv anketiranih, smo skupine učiteljev osebno poprosili za sodelovanje, jim na kratko pojasnili njen namen ter jim razdelili vprašalnike, ki so jih izpolnili individualno doma ali na kraju samem. Vprašalniku smo priložili pisemsko ovojnico z naslovom in poštno znamko. Vrnjenih je bilo 73,9 % poslanih vprašalnikov.

Analiza podatkov

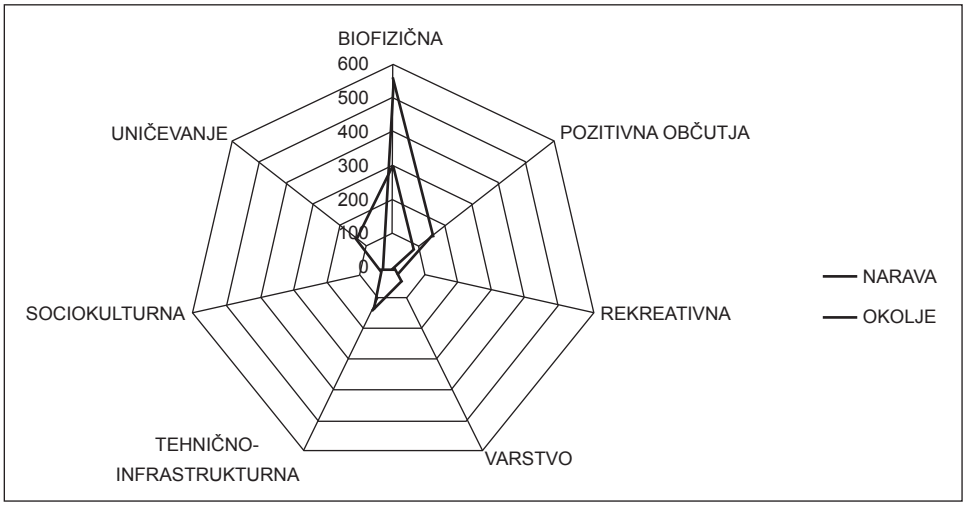
Pri analizi prostih asociacij je bil izbran kvalitativen pristop po metodi analize vsebine (Sagadin, 1993). Dobesedne transkripcije odgovorov so bile osnova za identifikacijo glavnih dimenzij, na tej osnovi je bila izdelana shema za kodiranje odgovorov. Pri oblikovanju dimenzij konceptov narava in okolje smo se zgledovali po raziskavi Flogaitis in Agelidou (2003). V vsaki kategoriji so bili odgovori prešteti in številčno prikazani v tabelah. Ko je šlo za veliko število pojmov, ki so imeli nizko frekvenco, vendar so se pomensko skladali s splošnejšim (nadrednim) pojmom z visoko frekvenco, smo pojme združevali. Npr. splošnejši in nadrejeni pojem *živali* tako vključuje različne vrste živali. Če pa je bila frekvenca določene vrste ali skupine živali 3 ali večkratna, smo jo uvrstili kot samostojno predstavo (npr. ptice). Pri tem sta poleg avtorja sodelovala tudi dva izkušena sodelavca, ki sta se seznanila z materialom za analizo in s katerima so bili prediskutirani postopki analize ter oblikovanja dimenzij.

Rezultati in diskusija

Posamezne predstave in njihove frekvence ter frekvence dimenzij so predstavljene v tabeli 1 (narava) in tabeli 2 (okolje). Primerjava posameznih dimenzij besed okolje in narava je tudi grafično prikazana (Slika 1).

Od skupno 885 predstav učiteljev biologije o naravi je bilo 816 (92,2 %) predstav razvrščenih v sedem kategorij (dimenzij). Preostalih 7,8 % predstav, ki jih nismo uspeli kategorizirati, ni vključenih v tabelo 1. Iz tabele je razvidno, da učitelji biologije najpogosteje omenjajo *biofizično dimenzijo* narave, ki jo sestavljajo elementi žive in nežive narave, sistemi in procesi. Med živalmi so daleč najpogosteje omenjene ptice. Med rastlinami imajo to mesto drevesa. Redko so omenjeni znanstveni pojmi, kot je ekologija, biodiverziteta in biotop. V *biofizični dimenziji* prevladujejo konkretni elementi žive narave. Učitelji biologije velikokrat naravo povežejo tudi z vodnimi ekosistemi (npr. morje, potok, reka, jezero, slap). Anketirani učitelji omejujejo uporabo besede narava na deskriptivno raven, kar ugotavljata tudi Flogaitis in Agelidou (2003).

Druga najpogosteje omenjena je *dimenzija kvalitet in ugodja*. Narava vprašanim predstavlja prostor miru, tišine, sprostitve, lepote, čistosti, svežine, zdravja, veselja, sreče, počitka, uživanja, energije itd. Kaplan (1995) ugotavlja, da vsaka dovolj intenzivna, stresna in dolga naloga lahko postopoma pripelje posameznika do upada in izgube pozornosti, zato je za oddih od neposredne pozornosti potrebno poiskati nekaj, kar je samo po sebi privlačno in ne zahteva pretiranega napora. Kahn (2001) in Kaplan (1995) ugotavljata, da ima narava obilo privlačnih objektov, ki brez veliko



Slika 1: Dimenzije pojmovanja okolja in narave
Fig. 1: Conceptual Dimensions of Environment and Nature

truda pridobijo našo pozornost. V naravi naj bi se zato počutili prijetno, se spočili in napolnili z energijo.

Med pogosteje omenjene dimenzije predstav sodi *rekreativna dimenzija*. Učitelji omenjajo sprehode in izlete v naravo, različne oblike športa in rekreacije. V *sociokulturni dimenziji* je edina predstava človek. V *dimenziji uničevanja in degradacije* omenjajo štiri predstave: onesnaževanje narave, posegi v naravo, uničenje in smeti. *Tehnično-infrastrukturna dimenzija* poudarja elemente človekovih bivališč in inftastrukture, kot so kmetija, poti in naselje. Kar pa zadeva varstvo narave so predstave učiteljev omejene na najbolj splošne pojme, kot so varstvo, ohranjanje, zavarovanje in skrb za naravo. Analiza predstav o pojmu narava je potrdila ugotovitve Mausnerja (1996), Flogaitisa in Agelidoua (2003) ter Krömkera (2004), da anketirani naravo najpogosteje povezujejo z različnimi vrstami živali in rastlin ter prostori, kjer ni stalno prisoten človek. Redkeje pa so omenjene dimenzija kvalitet in ugodja ter rekreativna dimenzija.

Od skupno 791 predstav učiteljev o okolju je bilo 732 (92,5 %) predstav razvrščenih v sedem kategorij (dimenzij). Preostalih 7,5 % predstav, ki jih nismo uspeli kategorizirati, ni vključenih v tabelo 2. Iz učiteljevih predstav o okolju je razvidno, da je najpogosteje omenjena *biofizična dimenzija* (Tabela 2). »Okolje« pogosto asociirajo z antropogenimi ekosistemi, kot so vrt, polje, travnik, park in sadovnjak. Redkeje pa omenjajo znanstvene pojme, kot so biodiverziteta, biocenoza, biotop in ekosistem. Izjema je le beseda ekologija, ki jo učitelji pogosto omenjajo. Glede na znanstveni pomen besede ekologija, ki se kot veda ukvarja s proučevanjem odnosov med organizmi in njihovim organskim in anorganskim okoljem, smo besedo uvrstili v biofizično dimenzijo. Menimo pa, da z ozirom na njeno zastopanost v predstavah o okolju, ter nezastopanostjo v predstavah o naravi, besedo ekologija predvsem povezujejo z okoljevarstveno problematiko.

Druga pomembnejša dimenzija je *dimenzijo uničenja ali degradacije,* kjer učitelji omenjajo onesnaževanje, smeti, uničenje, hrup, dim, utesnjenost, smrad in smog. Rezultati potrjujejo, da se pri razumevanju besede okolje vse bolj izpostavljajo okoljevarstveni problemi (MacDonald, 2003). To daje misliti, da danes okolje vse bolj povezujemo s človekovim zdravjem in kvaliteto bivanja.

Po frekvenci izstopajoča je tudi *tehnično-infrastrukturna dimenzija*. Učitelji pogosto omenjajo mesta, vasi, promet, zgradbe, naselja, ceste in industrijo. V manjši meri so omenjene *di-*

Tabela 1: Predstave učiteljev biologije o naravi.
Table 1: Biology Teachers' Conceptions about Nature.

Dimenzija	**Št. odgovorov**	**Posamezne predstave in število odgovorov**
biofizična dimenzija: različni elementi, sistemi in procesi narave	539	rastline (69), živali (69), gozd (43), zrak (36), voda (36), travnik (26), živo / neživo (30),zelenje (22), morje (20), sonce (20), gore (12), ptice (12), drevesa (11), življenje (10), potok (10), dež (10), reka (8), jezero (8), kamnine (8), ravnovesje (8), barve (7), polje (7), ekologija (7), hrana (6), ekosistem (6), biotop (5), sožitje (4), življenjski prostor (4), vrt (4),, gobe (4), veter (4), ptičje petje (4), zemlja (3), biodiverziteta (3) sneg (3)
dimenzija kvalitet in ugodja	152	mir in tišina (35), čisto (23), sprostitev (22), lepota (22), svežina (10), uživanje (7), počitek (6), življenjska energija (5), svoboda (4), ljubezen (3), spoštovanje in občudovanje (3), neokrnjena (3), zdravje (3), prijetne vonjave (3), veselje in sreča (3),
dimenzija uničevanja ali degradacije	40	onesnaževanje (25), posegi v naravo (6), uničenje (5), smeti (4)
rekreativna dimenzija: prosti čas v naravi	32	sprehodi in izleti (24), šport in rekreacija (8)
dimenzija varstva: oblike varstva narave	28	varstvo narave (11), ohranjanje (7), zavarovanje (6), skrb (4)
sociokulturna dimenzija	25	človek (25)
tehnično – infrastrukturna dimenzija: poudarja elemente človekovih bivališč in gradnje	8	kmetija (5), naselje (3)

menzija kvalitet in ugodja (npr. čistoča, urejenost, sprostitev, zdravje), *sociokulturna dimenzija* (npr. človek, dom, soseska), *dimenzija varstva* (npr. varovanje, skrb, ločevanje odpadkov, čistilne naprave…) in *rekreativna dimenzija* (npr. sprehodi, rekreacija).

Predstave o naravi in okolju se razlikujejo predvsem v frekvencah *biofizične dimenzije, dimenzije kvalitet in ugodja, tehnično-infrastrukturne dimenzije* ter *dimenzije uničevanja ali degradacije.* Iz grafičnega prikaza (Slika 1) je najbolj opazna razlika v frekvenci biofizične dimenzije v korist pojma narava, kar ugotavljajo tudi druge raziskave (Flogaitis in Agelidou, 2003; Sato in James, 1999). V biofizični dimenziji okolja in narave so predstave zelo podobne. Malo je znanstvenih pojmov. Prevladujejo konkretni naravni elementi. Učiteljeva percepcija torej temelji predvsem na empiričnih izkušnjah in je le v manjši meri določena z znanstveno razlago pojmov.

Okolje je, v primerjavi z naravo, bolj razumljeno kot prostor, ki se podreja interesom človeka. V predstavah o okolju je bolj izražen vpliv človekovih dejavnosti, ki imajo uničujoč vpliv na okolje. Več je tudi različnih predstav v dimenziji uničevanja in degradacije. Opazno manjši je pomen okolja kot prostora, kjer se človek umiri, naužije lepot, spočije ali rekreativno udejstvuje. V svojih predstavah nekoliko večjo pozornost posvečajo varstvu okolja kot pa varstvu narave. Tudi samo število različnih predstav v dimenziji varstva je večje pri pojmovanju okolja.

Tabela 2: Predstave učiteljev biologije o okolju.
Table 2: Biology Teachers' Conceptions about Environment.

Dimenzija	Št. odgovorov	Posamezne predstave in število odgovorov
biofizična dimenzija: različni elementi, sistemi in procesi v naravi	302	naravno okolje (36), življenjski prostor (32), zrak (21), voda (21), ekologija (20), gozd (14), bivališče (13), rastline (13), živali (13), travnik (12), polje (12), vrt (11), biotop (10), reke (10), živo/neživo (11), ekosistem (7), morje (7), biocenoza (6), biodiverziteta (5), gore (5), drevesa (4), potok (4), park (3), zelenje (3), tla (3), sadovnjak (3), svetloba (3)
dimenzija uničevanja ali degradacije	134	onesnaževanje (54), smeti (33), hrup (17), smog in dim (7), ogroženo (7), kisel dež (5), umazanija (4), uničenje (4), utesnjenost (3)
tehnično – infrastrukturna dimenzija: poudarja elemente človekovih bivališč in gradnje	131	mesto/mestno (20), promet (20), zgradbe (20), ceste (15), industrija (13), druga infrastruktura (12), vas/vaško (9), naselje (8), beton (6), šola (4), urbano (4)
dimenzija kvalitet in ugodja	76	čistoča (45), urejenost (15), lepota (10), sprostitev (3), zdravje (3)
dimenzija varstva: oblike varstva okolja	43	varstvo (13), čistilne naprave (8), ohranjanje (5), skrb (4), osveščanje (4), zbiranje in sortiranje odpadkov (3), čistilne akcije (3), zavarovanje (3)
sociokulturna dimenzija	37	človek (26), dom (7), soseska (4)
rekreativna dimenzija: prosti čas v okolju	9	sprehodi in izleti (6), šport in rekreacija (3)

Zaključek

Pri naravi in okolju gre za dva kompleksna in hitro razvijajoča se pojma, ki dobivata vse bolj interdisciplinarni značaj, zato obstaja objektivna nevarnost, da učiteljevo poklicno omejeno in površno pojmovanje besede privede le-to do njene kolizije, ko nujno prehoden značaj pojmovanja dobi obliko neizpodbitne definicije. Pojmovanje besede okolje je namreč že po svoji osnovni opredelitvi (t.j. prostor, ki obdaja osebo ali stvar) zavezana k prehodnosti, ki jo narekujeta tako nosilec pojma okolje (človek) kot okolje samo. Temelječ na njenih osnovnih pojmovnih značilnostih, ki nenazadnje izvirajo že iz same teorije evolucije, lahko enako zaključimo za besedo narava.

Iz tega sledi zaključek, da je potrebno spremljati razvoj temeljnih pojmov, kot sta okolje in narava, ter jih znati spretno opredeljevati v luči globalnih (interdisciplinarnih) ciljev VITR. Tako stroka kot tudi učitelji sami bi morali spremljati razvoj temeljnih pojmov ter skrbeti za permanentno izobraževanje. Samo tako bo namreč učitelj sposoben učencem in dijakom dajati nastavke kritičnega mišljenja, ki je predpogoj in jedro VITR.

Summary

The UN's declaration of a Decade of Education for Sustainable Development (2005–2014) was clearly a big step in encouraging sustainability in our society and also a big step in stressing the importance of Education for Sustainable Development (ESD). White Paper on Education in the Republic of Slovenia (1996) emphasised the importance of education for healthy lifestyle and development of a responsible relationship with the environment and nature. Therefore educators, such us biology teachers, have an important responsibility to implement the ESD in everyday school practice.

In this article we explored biology teachers' concepts and ideas about nature and the

environment, two basic concepts in ESD. The study involved 105 biology teachers working in primary schools in Slovenia. The participants completed a word association questionnaire. Each participant wrote down up to ten associated words for "nature" and the "environment". When the participants completed the questionnaire, an analysis of the data generated from associated words was carried out. For the analysis we followed the Flogaitis in Agelidou (2003) example. First, all the associated words used by the participants were counted. Associated words with the same meaning were then coded together. Low-frequency words with the same meanings as high-frequency words were subsumed within the more frequent (general) ones. The words were then categorized, using as a criterion their semantic relationship, and the frequency of the words in each category was calculated. The associated words for environment and nature were grouped into seven categories.

The analyses of the data showed that the dominant dimension of biology teachers concerning nature was biophysical. It was limited to descriptive considerations based on the more obvious "natural" elements. Scientific concepts, such as ecology, biodiversity and biotope, were rarely mentioned by participants. Nature was seen as non-human environment used by humans only as place of sport, recreation, beauty, quietness and rest. When we analyzed the results for the environment and compared them with those for nature, we could discover that the biophysical dimension was also the dominant one. Dominant dimensions of environment were also the dimension of destruction and the technostructure dimension. Environment was seen as a place dominated by human activities, the infrastructure and the consequences of pollution and degradation. Some similarities in teachers' concepts of nature and environment indicate conceptual confusion. The biophysical dimensions were regarded as almost the same within the two concepts, as they had similar variety of words.

These findings should be taken into consideration while planning in-service programs for biology teachers. Nature and environment are two complex and quickly developing concepts. They are gaining on interdisciplinary character; therefore it is important that teachers follow the development of basic ESD concepts.

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Sprejeto (accepted):

The Sensitivity of two biochemical biomarkers in berrestrial isopods after short-term copper exposure

Občutljivost dveh biokemijskih biomarkerjev v kopenskih rakih po kratkotrajni izpostavitvi bakru

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Abstract: Biochemical biomarkers, e.g. enzyme activities, have been traditionally considered a very sensitive and specific tool to characterize the hazard of pollutants to organisms. Among them, considerable attention was given to antioxidant enzymes catalase and glutathione S-transferase, which respond to changes in the quantity of reactive oxygen species. In the present study, the two enzymes were assessed in terrestrial isopods *Porcellio scaber* acutely (3 days) exposed to redox active copper and compared to their whole-organism responses, such as feeding, weight change and survival. The animals were fed with copper contaminated food for 3 days and afterwards for another 3 and 6 days with uncontaminated food. In contrast to expectations, no changes of antioxidant enzymes were found throughout the experiment, while feeding parameters were already decreased after 3 days of exposure at the highest exposure concentration 5000 μg/g dry food. The concentrations tested were not acutely lethal for isopods and did not affect their weight change. These findings imply that biochemical biomarkers in some cases are not a fast and sensitive measure to characterise the hazard of chemicals. The observed finding is probably the result of interplay between a very short time of exposure and the type of chemical chosen. Namely, a special relationship exists between isopods and copper, since it is an essential element for *P. scaber*. It is recommended that more data on the relationship between lower and higher-level biomarkers in isopods after different exposure periods is needed and this knowledge will increase their relevance in future studies on the hazard of new emerging contaminants.

Keywords: antioxidant enzymes, biomarker; catalase; environmental risk assessment; glutathione S-transferase; hazard, *Porcellio scaber*

Introduction

The number of environmental studies employing biomarkers (e.g. biological responses that provide a measure of exposure and/or effect of chemicals) as indicators of chemical stress has increased significantly since the beginning of 1990's. Pursuant to the assumption that pollutants have an impact on cellular levels before their effects are observed at the whole-organism level, biochemical biomarkers (e.g. the activities of enzymes) were considered one of the most promising (Adams 2002). Since biochemical response is dependent upon the interaction of the toxicant with a molecular target, it was expected that biochemical biomarkers could be used to determine the bioavailability of absorbed toxicant and could help to identify causal mechanisms potentially responsible for effects realized at higher levels of organization (Peakall & Walker 1994). Biochemical biomarkers were considered a very sensitive (responding already at very low concentration of a toxicant) and specific measure (responding only to a specific class of chemicals).

Also, it is expected, that the changes of biochemical biomarkers are preceded by effects at a higher level of biological organization including processes such as growth, reproduction and mortality (Depledge & Fossi 1994).

In the present paper, biochemical biomarkers catalase and glutathione-S-transferase and whole-organism responses of adult terrestrial isopods *Porcellio scaber* Latreille 1804 (Isopoda, Crustacea) exposed to copper were investigated. Toxicity testing with these invertebrates has been recognized as fast, routine and inexpensive and has been commonly applied to identify hazard of different metals and pesticides (Drobne & Hopkin 1995, Drobne & al. 2008). Terrestrial isopods are commonly regarded as suitable test organisms for metal exposure, because they are able to accumulate high amounts of metal ions (Hopkin 1989). Their main metal storage and metabolic organ is digestive midgut gland (hepatopancreas), where most metal is deposited in intracellular granules. Several responses along biological complexity of these animals were proposed as toxicological end-points: moult frequency (Drobne & Štrus 1996), food consumption, food selection, behavioural responses (Zidar & al. 2003), gut microflora (Drobne & al. 2002), and epithelial thickness (Lešer & al. 2008). However, despite the wide applicability of isopods in toxicity testing, biochemical biomarkers have not been sufficiently investigated and employed after different exposure periods.

The two biochemical biomarkers chosen in the present work were antioxidant enzymes catalase (CAT) and glutathione-S-transferase (GST). Catalase is a very highly conserved enzyme that has been identified in most organisms, including vertebrates, invertebrates, plants, fungi and bacteria. It is primarily localized in peroxisomes, where many enzymes generate hydrogen peroxide, which is further degraded by CAT. Glutatione-S-transferases constitute a large family of multifunctional enzymes involved in the cellular detoxification of many physiological and xenobiotic substances. Their role is to render water-soluble glutathione conjugates, thus facilitating their elimination. Besides biotransformation of xenobiotics, they also detoxify endogenous products formed during lipid peroxidation and are therefore also regarded as antioxidant enzymes (Halliwell & Gutteridge 2007).

Copper (Cu^{2+}) was selected as a model toxicant due to its presumed production of reactive oxygen species and induction of oxidative stress damages, such as the effects on the DNA integrity and the induction of lipid peroxidation (Stohs & Bagchi 1995). It is expected, that the activities of both antioxidant enzymes CAT and GST will increase upon exposure to Cu^{2+}. Copper is a common environmental soil pollutant, although recent concentrations detected in topsoil of different European towns (up to 290 µg/g) (Poggio & al. 2008) were not as high as in the past (industrial site; up to 15000 µg/g) (Bengtsson & Tranvik 1989). Very high concentrations of Cu^{2+} up to 2700 µg/g are still being reported in mine wastes (Perez-Lopez & al. 2008).

The accumulation, metabolism, storage, detoxification, excretion and toxicity of Cu^{2+} in isopods are well documented (Hopkin 1989). Terrestrial isopods exhibit high tolerance towards copper, since they are able to accumulate vast amounts of this element in cuprosomes, vesicles of the lysosomal system and epithelial cells of hepatopancreas (reviewed in Weissenburg & Zimmer 2003). The reason for storage lies in the fact, that copper is an essential metal in isopods, because it is a part of the oxygen carrying protein haemocyanin, it promotes digestive processes of leaf litter and is involved in detoxification processes and immune response of isopods (Irmak & al. 2005).

While copper is essential for isopods, it may become toxic at high concentrations (Hassall & Rushton 1982, Hopkin 1989, Farkas & al. 1996, Zidar & al. 2003). In these toxicity studies mainly long exposure periods up to 6 weeks were applied. In the present paper, a very short exposure period (3 days) was selected due to presumably quick response of biochemical biomarkers. It has been previously shown, that this period is long enough to cause sublethal effects of heavy metals on digestive glands in isopods (Nolde & al. 2006).

The aim of the present paper was to assess the short-term effects of Cu^{2+} on biochemical biomarkers and whole-organism (higher-level) responses, such as feeding, weight change and survival of isopods *P. scaber*. The sensitivity of biochemical biomarkers in comparison to higher-level responses is discussed.

Materials and methods

Chemicals

The following chemicals were purchased from Sigma-Aldrich (Munich, Germany): dibasic and monobasic potassium phosphate, hydrogen peroxide (30%), 1-chloro-2,4-dinitrobenzene, L-glutathione (reduced form). Protein Assay Reagents A and B were purchased from Pierce (Rockford, IL, USA). The source of Cu^{2+} ions was $Cu(NO_3)_2$ (Merck, New Jersey, USA). All chemicals were of the highest commercially available grade, typically 99% or higher.

Test organisms

Terrestrial isopods (*Porcellio scaber*, Latreille 1804) were collected under the litter layer in an uncontaminated location in the vicinity of Ljubljana. The experiments were conducted within 11 days after the collection of animals from the field, as previously proposed by Jemec & al. (2008). In the laboratory, the animals were kept in a terrarium (20 × 35 × 20 cm) filled with a 2 to 5 cm layer of moistened sand and soil and a thick layer of partly decomposed hazelnut tree leaves (*Corylus avellana*). The substratum in the terrarium was heated to 80°C for several hours to destroy predators (spiders) before the introduction of the isopods. The culture was kept at controlled room temperature (21±1°C), 16:8 h light/dark regime and high humidity.

Feeding experiments

The adults of *P. scaber* with body weights ranging from 30 to 80 mg, of both sexes and all moult stages, were used in the experiment. Also, females with brood chamber were used in the experiments, but these specimens were not included in the analyses of whole-organism endpoints, because both the moult and the presence of broods might influence the feeding and animal mass change. However, all animals were included in the analysis of CAT and GST, since these biomarkers were previously found independent on these endogenous characteristics of isopods (Jemec & al. 2008). Each animal was placed individually in a Petri dish, to which individual pieces of dry leaves dosed with Cu^{2+} were added. Humidity in the Petri dishes was maintained by regular spraying with tap water on the internal side of the lids. All Petri dishes were placed in a large plastic-covered glass container maintained at relative humidity close to 100%, and a 16:8 h light/dark regime.

The food for the feeding experiment was prepared following the protocol proposed by Drobne & Hopkin (1995) and in detail described by Jemec & al. (2008). Different Cu^{2+} solutions were spread over the dry leaves using a brush in a way that they contained 100, 1000 or 5000 µg of Cu^{2+} per gram dry weight of leaves The amount of copper on leaves was not measured in this particular study, since our previous experience with this kind of food preparation show, that the actual concentrations of Cu^{2+} on leaves are within 5 % of the nominal ones (Zidar & al. 2003; Zidar & al. 2004). The concentrations were selected based on previous toxicity testing with this organism (Zidar & al. 2003). The control was prepared by applying distilled water instead of Cu^{2+} dispersion.

The feeding experiment consisted of 3 days feeding with Cu^{2+} contaminated food, followed by 3 days and 6 days period of feeding without contaminated food (e.g. recovery). In each exposure group 18 animals was exposed at the beginning of the experiment. After 3 days of feeding 6 animals were removed for enzyme analysis, 12 of them were fed on non-contaminated food. After 3 days of recovery 6 animals were removed and after 6 days of recovery the remaining 6 were used to measure the enzymes. This experiment was repeated twice, so altogether 12 animals per concentration and a certain time exposure (3 days feeding, 3 days recovery, 6 days recovery) were exposed. After each exposure period whole-organism responses were evaluated. The leaves were weighed after drying at room temperature for 24 h, and the faecal pellets were counted and weighted after drying in the exsiccator for 48 h. The animals were weighted right after the experiment, they were dissected and the digestive glands were isolated for measurements of CAT and GST activities. Animal mortality was also recorded.

Measurements of biochemical biomarkers

The whole digestive gland of each animal was homogenized in 800 µL of 50 mM phosphate buffer pH 7.0 for 3 min. The homogenate was centrifuged for 15 min at 15000 *g* and 4 °C. The GST activity was measured on microtiter plates (Bio-Tek® Instruments, USA; PowerWave™ XS) using 1-chloro-2.4-dinitrobenzene as a substrate (Jemec & al. 2008). The GST activity was expressed in nmoles of conjugated GSH/min/mg protein (extinction coefficient ε_{340} = 9600 $M^{-1}cm^{-1}$). The CAT activity was determined as described in Jemec & al. 2008. The reaction was followed spectrophotometrically on a Shimadzu UV-2101PC spectrophotometer (Japan). The CAT activity was expressed in µmoles of degraded hydrogen peroxide/min/mg protein (extinction coefficient ε_{240} = 43.6 $M^{-1}cm^{-1}$). Protein concentration was measured using a BCA™ Protein Assay Kit, a modification of the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA).

Data analysis

The feeding rate and a defecation rate of isopods were calculated as the mass of consumed leaf and mass of faecal pellets per animal wet weight and per day, respectively. The animal mass change was determined as the difference in animal mass at the beginning and at the end of the experiment.

For statistical analysis and the presentation of results, both experiments were combined, because no differences between the controls and corresponding concentrations of both experiments were observed. In Fig.1 data for 12 animals are shown at each concentration. In result on feeding parameters (Fig.2.) the total number of animals included at each concentration is: 36 after 3 days of feeding, 24 after 3 days of recovery and 12 after 6 days of recovery. Different numbers of animals are due to gradual animal removal for enzyme analysis. The significant differences between the control and exposed groups of animals were determined by Kruskal-Wallis analysis and Mann-Whitney *U* test ($p<0.05$) using Statgraphics software (Statgraphics Plus for Windows 4.0, Statistical Graphics, Herndon, VA, USA). Homogeneity of variance was tested using Levene's test.

Results

The activities of antioxidant enzymes

The activities of both antioxidant enzymes CAT and GST remained unchanged in digestive glands of isopods exposed up to 5000 µg Cu^{2+}/g dry food for 3 days. Also, no changes in comparison to control were observed after 3 days and 6 days of recovery without contaminated food (**Fig. 1 a-f**).

The activities of CAT and GST in control animals after 6 days of recovery were significantly lower as in controls after 3 days of feeding, which is in line with our previous observation that the activities of both enzymes decrease while the animals are kept in the laboratory (please see the explanation in Jemec & al. 2008). In our case, the animals investigated after 3 days of feeding were kept in the laboratory for 3 days, while the ones dissected after 6 days of recovery were kept for 9 days. Since all the specimens exposed within a certain experimental group (3 days feeding, 3 days recovery, or 6 days recovery) were kept in the laboratory for the same period of time, the comparisons between the control and exposure concentrations in each experimental group were possible.

Whole-organism parameters

After 3 days of exposure, the feeding rate (W = 308; p=0.0039) and defecation rate (W=359, p=0.0270) were decreased at 5000 µg Cu^{2+}/g dry food. After 3 days of recovery feeding and defecation rates were not changed, but they were increased at all three exposure concentrations of Cu^{2+} after 6 days of recovery (Feeding rate: W=77, p=0.0403 at 100 µg/g; W = 81, p=0.0185 at 1000 µg/g; W=53, p=0.0262 at 5000 µg/g and defecation rate: W=73, p=0.0186 at 100 µg/g; W=79, p=0.0044 at 1000 µg/g; W=55, p=0.0181 at 5000 µg/g) (**Fig. 2 a-f).** No weight change of isopods or their mortality was observed after 3 days feeding with Cu^{2+} dosed food (p>0.05), and 3 days or 6 days of feeding with uncontaminated food.

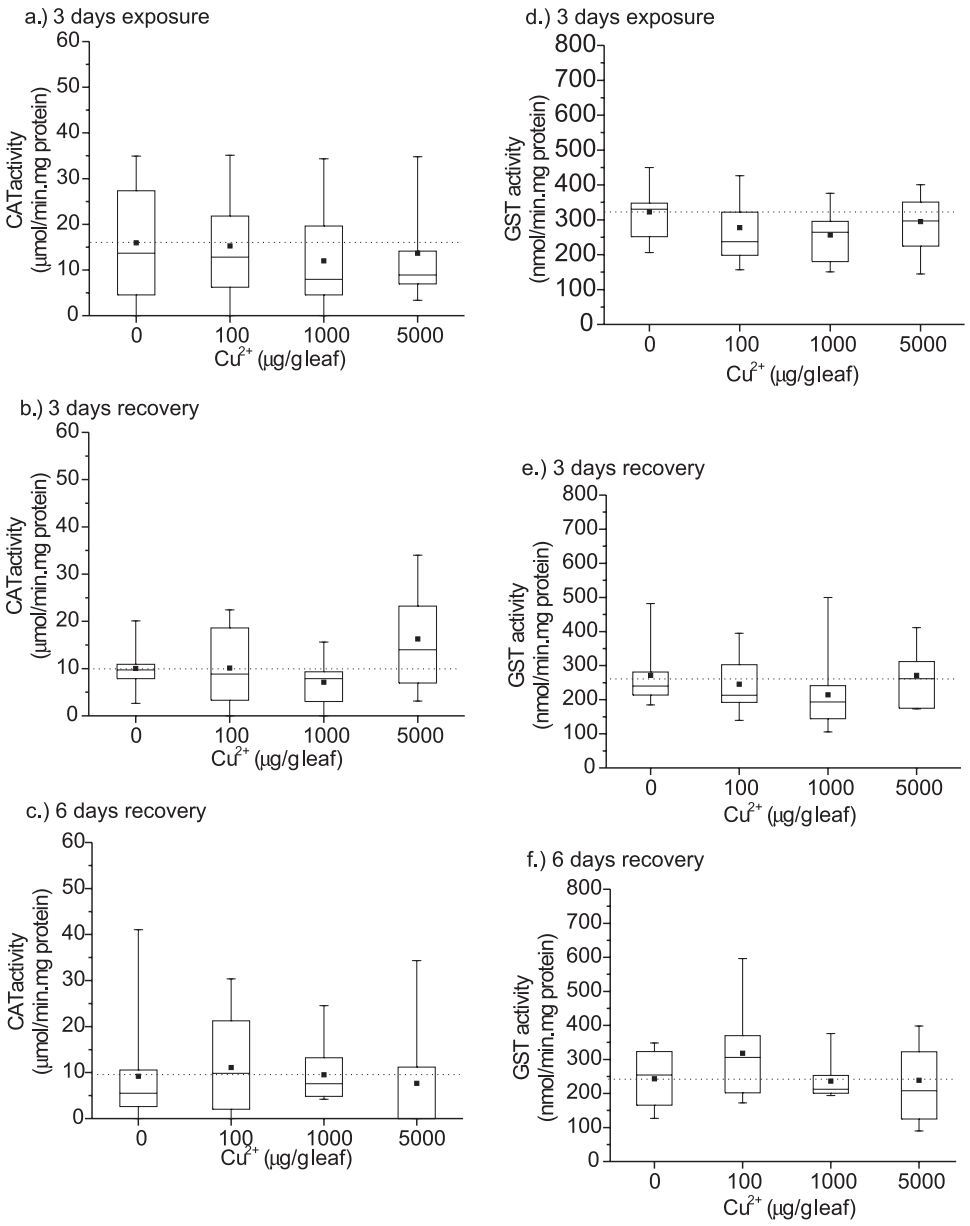

Fig. 1: The effect of Cu^{2+} on the activities of catalase and glutathione-S-transferase after 3 days of exposure, 3 days recovery and 6 days recovery. Symbols on the box plot represent: maximum and minimum value (whiskers: ⊥), mean value (■). The dashed line represents the mean value of the control.

Slika 1: Učinki Cu^{2+} na aktivnosti katalaze in glutathione S- transferaze po 3 dneh hranjenja z onesnaženo hrano ter 3 in 6 dneh hranjenja z neonesnaženo hrano. Simboli pomenijo: maksimalna in minimalna vrednost (⊥), srednja vrednost (■). Črtkana črta predstavlja srednjo vrednost kontrole.

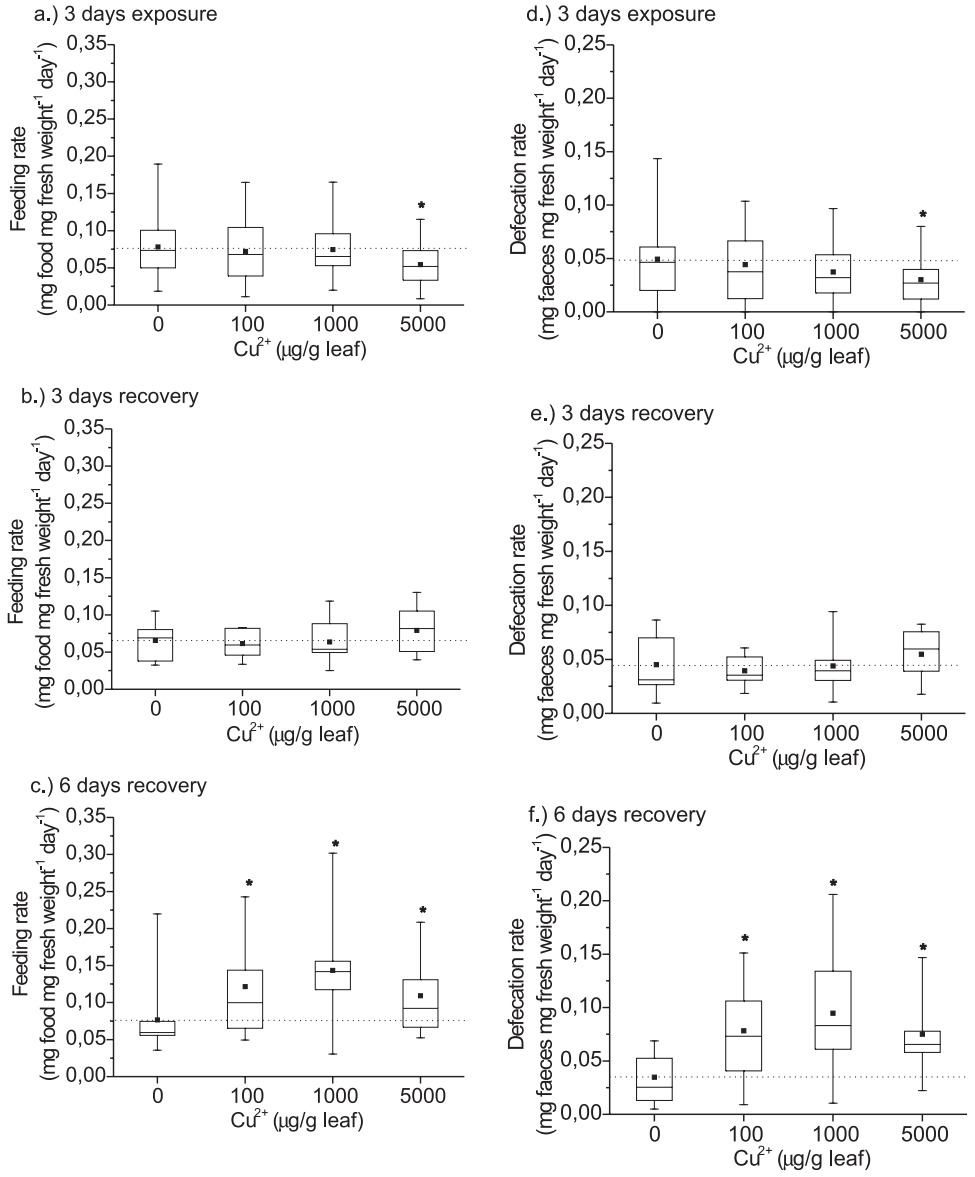

Fig. 2: The effect of Cu^{2+} on feeding rate and defecation rate after 3 days exposure, 3 days and 6 days recovery. Symbols on the box plot represent: maximum and minimum value (whiskers: ⊥), mean value (■). The dashed line represents the mean value of the control. The effects at a certain exposure concentration, which are significantly different in comparison to control, are shown (*) $p<0.05$.

Slika 2: Učinki Cu^{2+} na hranjenje in iztrebljanje kopenskih rakov po 3 dneh hranjenja z onesnaženo hrano ter 3 in 6 dneh hranjenja z neonesnaženo hrano. Simboli pomenijo: maksimalna in minimalna vrednost (⊥), srednja vrednost (■). Črtkana črta predstavlja srednjo vrednost kontrole. Statistično značilni različni učinki glede na kontrolo so označeni z (*) ($p<0.05$).

Discussion

Biochemical biomarkers are generally considered to be more sensitive than whole-organism responses which means, that the changes at the sub-cellular level should be detected at lower concentrations than the ones at the whole-organism level. However in this study no changes of biochemical biomarkers were observed, while changes in whole-organism parameters, such as feeding behaviour, were noticed. The literature contains many such examples where biomarker responses did not become detectable until the exposure levels at which already reductions in survival, growth and reproduction became apparent (Brown & al. 2004; Forbes & al. 2006). It has previously been suggested, that this is probably due to transient nature of biomarker response and their high response variability, which depends on the species being investigated, the periods of exposure, and the type of chemicals chosen (Barata & al. 2005).

The relationship between biochemical biomarkers and whole-organism responses obtained in this paper is therefore most probably a result of interplay between a very short period of exposure and the type of chemical chosen. Namely, a special relationship exists between isopods and copper, since it is an essential element for *P. scaber*. A very complex process appears to be involved in balancing between copper nutritional requirements and mitigation from copper toxic effects. Apart from the physiological mechanisms that enable assimilation and excretion of this metal, isopods are known to regulate copper intake at the behavioural level by discriminating between highly copper contaminated food and uncontaminated diet (Weissenburg & Zimmer 2003, Zidar & al. 2004). Several mechanisms, by which isopods could discriminate copper, were proposed. Among them are the existence of contact-chemoreception of copper (Weissenburg & Zimmer 2003) and it was also suggested that this discrimination might not necessarily be directly related to copper, but to copper induced changes in odour of metabolites released by microorganisms that colonise food particles. On the other hand, ingested copper might have adverse metabolic effects on isopods, which might reduce their consumption. Most probably both mechanisms are involved in the copper avoidance behaviour (Zidar & al. 2004).

The results of the present study show that the concentrations up to 5000 µg Cu^{2+}/g dry food were not lethal for isopods after 3 days of exposure and did not affect their weight change. However, feeding and defecation rate were already decreased at the highest concentration tested. These data cannot be directly compared to previous studies since there are not much literature data available after such a short exposure period. Another isopod species *Porcellionides pruinosus* exhibited avoidance behaviour when applied on soil contaminated with Cu^{2+} for 48 h (EC_{50} = 802 µg/g dry soil) (Loureiro & al. 2005). Isopods *P. scaber* consumed significantly less food as unexposed animals when exposed to 1200 µg of Cu^{2+}/g dry food weight for two weeks (Zidar & al. 2003), to 500 µg of Cu^{2+}/g dry food weight for 4 weeks (Farkas & al. 1996), and to 282 µg of Cu/g dry food weight for 6 weeks (Hassall & Rushton 1982). The specimens of *Porcellionides pruinosus* decreased food consumption after two weeks of feeding on 13710 µg of Cu^{2+}/g dry food and decreased their egestion rate at 6310 µg of Cu^{2+}/g dry food (Loureiro & al. 2006). After 6 days of recovery on non-contaminated food we noticed an increase of feeding at all three exposure concentrations of Cu^{2+}. The enhanced feeding of isopods after cessation of stress has been observed before (Drobne 1996) and is most probably a compensation for reduced consumption during copper exposure.

Although the link between the two groups of biomarkers (biochemical and higher level) was not established in this work, we believe that more data on the relationship between lower and higher-level biomarkers in isopods investigated after different exposure periods will increase their relevance in future studies on the hazard of new emerging contaminants, such as nanomaterials. Most probably, other novel biomarkers besides enzyme activities, such as proteomic and genomic profiles, will have a role in assessing risks associated with new contaminants.

Povzetek

Biokemijski biomarkerji, med katere uvrščamo aktivnosti encimov, so domnevno zelo občutljiva in specifična orodja pri določanju vplivov onesnaževal na organizme. Začetke njihove uporabe v okoljskih študijah beležimo okoli leta 1990, število znanstvenih objav z njihovo uporabo pa vse odtlej močno narašča. Med bolj pogosto uporabljenimi biokemijskimi biomarkerji sta antioksidativna encima katalaza in glutation S-transferaza, katerih aktivnost se spremeni v prisotnosti reaktivnih kisikovih zvrsti. V tej raziskavi smo proučevali aktivnosti omenjenih dveh encimov v prebavni žlezi kopenskih rakov enakonožcev *Porcellio scaber* ter spremembe na višjem organizacijskem nivoju kot so prehranjevanje, sprememba mase in preživetje. Ti testni organizmi so pogosto uporabljeni v toksičnih študijah za določanje tveganja različnih kovin in pesticidov, vendar pa je na voljo malo znanja o odzivih biokemijskih biomarkerjev po različnih časih izpostavitve. Kot modelno kemikalijo smo izbrali baker, za katerega je znano, da v organizmu povzroči nastanek reaktivnih kisikovih zvrsti. Dosedanje študije o vplivih bakra na te rake so potekale dalj časa (do 6 tednov), v tej raziskavi pa smo izbrali krajši čas (3 dni) zaradi pričakovanega hitrega odziva te vrste biomarkerjev. Poleg tega so nekatere prejšnje študije pokazale, da je ta čas že dovolj za povzročitev celičnih sprememb v prebavni žlezi kopenskih rakov. Organizme smo 3 dni izpostavljali hrani z dodanim bakrom, nato smo le to nadomestili z neonesnaženo hrano in opazovali živali še 3 in 6 dni po zamenjavi hrane. V nasprotju z našimi pričakovanji tekom poskusa nismo opazili sprememb aktivnosti antioksidativnih encimov, medtem ko so bili parametri na nivoju organizma spremenjeni. Pri najvišji testirani koncentraciji bakra (5000 μg/g suhe teže hrane) smo namreč opazili zmanjšano prehranjevanje in iztrebljanje rakov, ta koncentracija pa ni vplivala na njihovo težo in preživetje. Ti rezultati nakazujejo, da biokemijski biomarkerji v določenih primerih niso enostavna, hitra in nedvomno občutljiva mera za določanje tveganja kemikalij. Opaženi zaključki so najverjetneje posledica skupnega učinka zelo kratke dobe izpostavitve in izbranega tipa kemikalije. Baker je namreč esencialna kovina za rake *P. scaber*, zato imajo le ti kompleksne mehanizme regulacije ravnotežja količin bakra, med tistimi, ki so potrebne za normalno fiziološko delovanje ter tistimi, ki so za organizem že škodljive. Predlagam nadaljnje študije o povezavi med biomarkerji na nižjih in višjih nivojih biološke organizacije v kopenskih rakih pri različno dolgih časih izpostavitve, kar bo v prihodnosti povečalo njihov pomen v raziskavah o tveganjih kemikalij z neznanim delovanjem, kot so npr. produkti nanotehnologij.

Acknowledgements

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NAVODILA AVTORJEM

1. Vrste prispevkov

a) ZNANSTVENI ČLANEK je celovit opis originalne raziskave in vključuje teoretični pregled tematike, podrobno predstavljene rezultate z diskusijo in sklepe ter literaturni pregled: shema IMRAD (Introduction, Methods, Results And Discussion). Dolžina članka, vključno s tabelami, grafi in slikami, na sme presegati 15 strani; razmak med vrsticami je dvojen. Recenzirata ga dva recenzenta.

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d) KONGRESNA VEST seznanja bralce z vsebinami in sklepi pomembnih kongresov in posvetovanj doma in v tujini.

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Teksti naj bodo pisani v angleškem jeziku, izjemoma v slovenskem, če je tematika zelo lokalna. Kongresne in društvene vesti so praviloma v slovenskem jeziku.

4. Naslov prispevka

Naslov (v slovenskem in angleškem jeziku) mora biti kratek, informativen in razumljiv. Za naslovom sledijo imena avtorjev in njihovi polni naslovi (če je mogoče, tudi štev. faxa in e-mail).

5. Izvleček – Abstract

Podati mora jedrnato informacijo o namenu, uporabljenih metodah, dobljenih rezultatih in zaključkih. Primerna dolžina za znanstveni članek naj bo približno 250 besed, za kratko notico pa 100 besed.

6. Ključne besede – Keywords

Število naj ne presega 10 besed, predstavljati morajo področje raziskave, predstavljene v članku. Člankom v slovenskem jeziku morajo avtorji dodati ključne besede v angleškem jeziku.

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Nanašati se mora le na tematiko, ki je predstavljena v članku ali kratki notici.

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Tabele in slike (grafi, dendrogrami, risbe, fotografije idr.) naj v članku ne presegajo števila 10, v članku naj bo njihovo mesto nedvoumno označeno. Ves slikovni material naj bo oddan kot fizični original (fotografija ali slika). Tabele in legende naj bodo tipkane na posebnih listih (v tabelah naj bodo le vodoravne črte). Naslove tabel pišemo nad njimi, naslove slik in fotografij pod njimi. Naslovi tabel in slik ter legenda so v slovenskem in angleškem jeziku. Pri citiranju tabel in slik v besedilu uporabljamo okrajšave (npr. Tab. 1 ali Tabs. 1–2, Fig. 1 ali Figs. 1–2; Tab. 1 in Sl. 1).

9. Zakjučki

Članek končamo s povzetkom glavnih ugotovitev, ki jih lahko zapišemo tudi po točkah.

10. Povzetek – Summary

Članek, ki je pisan v slovenskem jeziku, mora vsebovati še obširnejši angleški povzetek. Velja tudi obratno.

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Uporabljene literaturne vire citiramo med tekstom. Če citiramo enega avtorja, pišemo Allan (1995) ali (Allan 1995), če sta dva avtorja (Trinajstić & Franjić 1994), če je več avtorjev (Pullin & al. 1995). Kadar navajamo citat iz večih del hkrati, pišemo (Honsig-Erlenburg & al. 1992, Ward 1994a, Allan 1995, Pullin & al. 1995). V primeru, če citiramo več del istega avtorja, objavljenih v enem letu, posamezno delo označimo s črkami a, b, c itd. (Ward 1994a,b). Če navajamo dobesedni citat, označimo dodatno še strani: Toman (1992: 5) ali (Toman 1992: 5–6). Literaturo uredimo po abecednem redu, začnemo s priimkom prvega avtorja, sledi leto izdaje in naslov članka, mednarodna kratica za revijo (časopis), volumen poudarjeno, številka v oklepaju in strani. Npr.:

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Ward J. V. 1994a: Ecology of Alpine Streams. Freshwater Biology 32 (1): 10–15.

Ward J. V. 1994b: Ecology of Prealpine Streams. Freshwater Biology 32 (2): 10–15.

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Toman M. J. 1992: Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1–7.

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The introduction must refer only to topics presented in the article or brief note.

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Articles should not contain more than ten (10) illustrations (graphs, dendrograms, pictures, photos etc.) and tables, and their positions in the article should be clearly indicated. All illustrative material should be provided as physical originals (photographs or illustrations). Tables with their legends should be submitted on separate pages (only horizontal lines should be used in tables). Titles of tables should appear above the tables, and titles of photographs and illustrations below. Titles of tables and illustrations and their legends should be in both Slovene and English. Tables and illustrations should be cited shortly in the text (Tab. 1 or Tabs. 1–2, Fig. 1 or Figs. 1–2; Tab. 1 and Sl. 1).

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Ward J. V. 1994a: Ecology of Alpine Streams. Freshwater Biology 32 (1): 10–15.
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